

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 May 2001 (25.05.2001)

PCT

(10) International Publication Number
WO 01/36610 A1

- (51) International Patent Classification⁷: C12N 9/64, 15/57, A61K 38/48, C12Q 1/68, G01N 33/50, C07K 16/40, C12Q 1/37, A61K 48/00
- (74) Agents: GOSMANN, Martin; Solvay Pharmaceuticals GmbH, Hans Böckler Allee 20, 30002 Hannover et al. (DE).
- (21) International Application Number: PCT/EP00/11532
- (22) International Filing Date:
17 November 2000 (17.11.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
99203862.0 19 November 1999 (19.11.1999) EP
1013616 19 November 1999 (19.11.1999) NL
00201937.0 31 May 2000 (31.05.2000) EP
1015356 31 May 2000 (31.05.2000) NL
- (81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (*for all designated States except US*): SOLVAY PHARMACEUTICALS B.V. [NL/NL]; C.J. Van Houtenlaan 36, NL-1381 CP Weesp (NL).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): DELEERSNIJDER, Willy [BE/BE]; c/o C.J. Van Houtenlaan 36, NL-1381 CP Weesp (NL). WIEGERS, Rico [NL/NL]; c/o C.J. Van Houtenlaan 36, NL-1381 CP Weesp (NL). WESKE, Michael [DE/DE]; c/o C.J. Van Houtenlaan 36, NL-1381 CP Weesp (NL).
- Published:
— With international search report.
— Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.
— Under Rule 91.1(f), with a request for rectification.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/36610 A1

(54) Title: HUMAN ENZYMES OF THE METALLOPROTEASE FAMILY

(57) Abstract: This invention relates to newly identified, polypeptides and polynucleotides encoding such polypeptides, which hereinafter are referred to as IGS5, to their use in therapy and in identifying compounds which may be stimulators and/or inhibitors which are potentially useful in therapy, and to production of such polypeptides and polynucleotides. The polypeptides and polynucleotides are related to the metalloprotease family and preferably of human type. The invention also relates to inhibiting or stimulating/activating the action of such polypeptides and polynucleotides, to a vector containing said polynucleotides and to a host cell containing such vector. The invention further relates to a method for screening compounds capable to act as a stimulator or inhibitor of said IGS5 enzyme. The Polypeptides of the present invention are of interest in the context of several dysfunctions, disorders or diseases. The Polypeptides of the present invention are in particular of interest in the context of cardiovascular diseases.

Novel Human Enzymes of the Metalloprotease Family

Description

FIELD OF THE INVENTION

This invention relates to newly identified polypeptides and polynucleotides encoding such polypeptides, to their use in therapy and in identifying compounds which may be stimulators and/or inhibitors which are potentially useful in therapy, and to production of such polypeptides and polynucleotides. More particularly, the polypeptides and polynucleotides of the present invention relate to enzymes which are members of the metalloprotease family of polypeptides or of particular families of structurally and functionally related polypeptides. These enzymes are hereinafter referred to as IGS5. The invention also relates to inhibiting or stimulating/activating the action of such polypeptides and polynucleotides, to a vector containing said polynucleotides and to a host cell containing such vector. The invention further relates to a method for screening compounds capable to act as a stimulator or inhibitor of said IGS5 enzymes.

BACKGROUND OF THE INVENTION

The drug discovery process is currently undergoing a fundamental revolution as it embraces "functional genomics", that is, high throughput genome- or gene-based biology. This approach as a means to identify genes and gene products as therapeutic targets is rapidly superceding earlier approaches based on "positional cloning". A phenotype, that is a biological function or genetic disease, would be identified and this would then be tracked back to the responsible gene, based on its genetic map position.

Functional genomics relies heavily on high-throughput DNA sequencing technologies and the various tools of bioinformatics to identify gene sequences of potential interest from the many molecular biology databases now available. There is a continuing need to identify and characterize further genes and their related polypeptides/proteins, as targets for drug discovery.

Among the polypeptides of interest in drug discovery there are metalloproteases and particular families of structurally and functionally related enzymes. Several diseases have been identified where metalloproteases play a critical role in the pathology of the

disease. For example, a number of zinc metalloproteases or particular families of structurally and functionally related enzymes have been identified and characterized in the state of the art, and it has become apparent that the participation of these enzymes, e.g. zinc metalloproteases, plays a role in a diverse array of biological functions encompassing both normal and disease situations. Zinc metalloproteases are subset of such enzymes whose catalytic functions are critically dependent on the zinc ion at the active site. This group of enzymes, which comprises various families classified on the basis of both sequence and structural information, are for example described to be intimately involved in such processes as e.g. embryonic development, cartilage and bone formation, processing of peptide hormones, reproduction, cardiovascular diseases, arthritis and cancer. Already active site-directed inhibitors of some of the zinc metalloproteases are being used therapeutically as e.g. antihypertensives.

On the basis of sequence and structural information around the zinc binding site of the zinc metalloproteases these enzymes may be classified into several families which may be further classified into superfamilies such as the "metzincins" (astacin, serratia, reprotin, matrixin), the "gluzincins" (thermolysin, neprilysin, angiotensin converting enzyme, aminopeptidase), or the "zincins" comprising the superfamilies of metzincins and gluzincins. Such grouping not only aids in the elucidation of common catalytic and biosynthetic processing mechanisms, but also is invaluable in elucidating the function(s) of newly identified proteins which possess similar zinc binding motifs. Some individual examples of metalloproteases, e.g. zinc enzymes, already identified in the state of the art comprise neprilysin, endothelin converting enzyme, angiotensin converting enzyme, thermolysin, aminopeptidase, astacin, serratia, reprotin, matrixin, insulinase, carboxypeptidase and DD-carboxypeptidase.

From the above evidence based on the state of the art it is apparent that metalloproteases and particular structurally and functionally related enzymes play key roles in health and disease. Thus there is a continued need to further uncover important functions and potential therapeutic applications for this group of enzymes and to provide novel metalloproteases with the subsequent development of novel synthetic stimulators (activators) or inhibitors, which can help to provide new treatments for a variety of diseases of socio-economic importance.

SUMMARY OF THE INVENTION

In one aspect, the present invention relates to IGS5, in particular to IGS5 polypeptides and IGS5 polynucleotides, preferably those related to the human species, to recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides, polynucleotides and recombinant materials, including the treatment of diseases in which polypeptides like metalloproteases or particular families of structurally and functionally related enzymes play a critical role in the pathology of the dysfunctions, disorders or diseases to be treated, hereinafter generally referred to as "the diseases". Examples of diseases, in context of which the use of the polypeptides and polynucleotides of the present invention is thought to be useful, include amongst others: CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophageal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as postoperative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus. In a further aspect, the invention relates to methods for identifying agonists and antagonists or inhibitors using the materials provided by the invention, and treating conditions associated with IGS5 imbalance with the identified

compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with inappropriate IGS5 activity or levels.

The Polypeptides of the present invention are in particular of interest in the context of cardiovascular diseases.

Table 1: IGS5-DNA ("IGS5DNA") of SEQ ID NO:1

5' -

TGCACCACCCCTGGCTGCGTGATAGCAGCTGCCAGGATCCTCCAGAACATGGACCCGACC
ACGGAACCGTGTGACGACTTCTACCAGTTTGCATGCGGAGGCTGGCTGCGGCGCCACGTG
ATCCCTGAGACCAACTCAAGATACAGCATCTTTGACGTCTCCGCGACGAGCTGGAGGTC
ATCCTCAAAGCGGTGCTGGAGAATTGACTGCCAAGGACCGGCCGGCTGTGGAGAAGGCC
AGGACGCTGTACCGCTCCTGCATGAACCAGAGTGTGATAGAGAAGCGAGGCTCTCAGCCC
CTGCTGGACATCTTGGAGGTGGTGGGAGGCTGGCCGGTGGCGATGGACAGGTGGAACGAG
ACCGTAGGACTCGAGTGGGAGCTGGAGCGGCAGCTGGCGCTGATGAACTCACAGTTCAAC
AGGCGCGTCCTCATCGACCTCTTCATCTGGAACGACGACCAGAACTCCAGCCGGCACATC
ATCTACATAGACCAGCCCACCTTGGGCATGCCCTCCCGAGAGTACTACTTCAACGGCGGC
AGCAACCGGAAGGTGCGGGAAGCCTACCTGCAGTTCATGGTGTCACTGGCCACGTTGCTG
CGGGAGGATGCAAACCTGCCCAGGGACAGCTGCCTGGTGCAGGAGGACATGATGCAGGTG
CTGGAGCTGGAGACACAGCTGGCCAAGGCCACGGTACCCCAGGAGGAGAGACACGACGTC
ATCGCCTTGTACCACCGGATGGGACTGGAGGAGCTGCAAAGCCAGTTTGGCCTGAAGGGA
TTTAACTGGACTCTGTTTATACAAACTGTGCTATCCTCTGTCAAAATCAAGCTGCTGCCA
GATGAGGAAGTGGTGGTCTATGGCATCCCCTACCTGCAGAACCTTGAAAACATCATCGAC
ACCTACTCAGCCAGGACCATAACAGAACTACCTGGTCTGGCGCCTGGTGCTGGACCGCATT
GGTAGCCTAAGCCAGAGATTCAAGGACACACGAGTGAACCTACCGCAAGGCGCTGTTTGGC
ACAATGGTGGAGGAGGTGCGCTGGCGTGAATGTGTGGGCTACGTCAACAGCAACATGGAG
AACGCCGTGGGCTCCCTCTACGTCAGGGAGGCGTTCCCTGGAGACAGCAAGAGCATGGTC
AGAGAACTCATTGACAAGGTGCGGACAGTGTTTGTGGAGACGCTGGACGAGCTGGGCTGG
ATGGACGAGGAGTCCAAGAAGAAGGCGCAGGAGAAGGCCATGAGCATCCGGGAGCAGATC
GGGCACCCTGACTACATCCTGGAGGAGATGAACAGGCGCCTGGACGAGGAGTACTCCAAT
CTGAACTTCTCAGAGGACCTGTACTTTGAGAACAGTCTGCAGAACCTCAAGGTGGGCGCC
CAGCGGAGCCTCAGGAAGCTTCGGGAAAAGGTGGACCCAAATCTCTGGATCATCGGGGCG
GCGGTGGTCAATGCGTTCTACTCCCCAAACCGAAACCAGATTGTATTCCCTGCCGGGATC
CTCCAGCCCCCTTCTTCAGCAAGGAGCAGCCACAGGCCTTGAACCTTTGGAGGCATTGGG
ATGGTGATCGGGCACGAGATCACGCACGGCTTTGACGACAATGGCCGGAACCTTCGACAAG
AATGGCAACATGATGGATTGGTGGAGTAACTTCTCCACCCAGCACTTCGGGAGCAGTCA
GAGTGCATGATCTACCAGTACGGCAACTACTCCTGGGACCTGGCAGACGAACAGAACGTG
AACGGATTCAACACCCTTGGGGAAAACATTGCTGACAACGGAGGGGTGCGGCAAGCCTAT

AAGGCCTACCTCAAGTGGATGGCAGAGGGTGGCAAGGACCAGCAGCTGCCCCGGCCTGGAT
 CTCACCCATGAGCAGCTCTTCTTCATCAACTACGCCAGGTGTGGTGCGGGTCTTACCGG
 CCCGAGTTTCGCCATCCAATCCATCAAGACAGACGTCCACAGTCCCCTGAAGTACAGGGTA
 CTGGGGTCGCTGCAGAACCTGGCCGCCTTCGCAGACACGTTCCACTGTGCCCCGGGGCACC
 CCCATGCACCCCAAGGAGCGATGCCGCGTGTGGTAG - 3'

Table 2: IGS5-protein ("IGS5PROT") of SEQ ID NO:2

CTTPGCVIAAARILQNMDPTTEPCDDFYQFACGGWLRRHVIPETNSRYSIFDVLRLDELEV
 ILKAVLENSTAKDRPAVEKARTLYRSCMNQSVIEKRGSQPLLDILEVVGGWPVAMDRWNE
 TVGLEWELERQLALMNSQFNRRVLIDLFIWNDDQNSSRHIIYIDQPTLGMPREYYFNGG
 SNRKVREAYLQFMVSVATLLREDANLPRDSCLVQEDMMQVLELETQLAKATVPQEERHDV
 IALYHRMGLEELQSQFGLKGFNWTLFIQTVLSSVKIKLLPDEEVVVYGI PYLQNLNIID
 TYSARTIQNYLVWRLVLDRIQSLQRFKDRVNYRKALFGTMVEEVRWRECVGYVNSNME
 NAVGSLYVREAFPGDSKSMVRELIDKVRTVFVETLDELGWMDEESKKKAQEKAMSIREQI
 GHPDYILEEMNRRRLDEEYSNLFSEDLFENSLQNLKVGARSLRKLREKVDPNLWIIGA
 AVVNAFYSPNRNQIVFPAGILQPPFFSKEQPQALNFGGIGMVIGHEITHGFDDNGRNFDK
 NGNMMDWWSNFSSTQHFREQSECMYQYGNYSWDLADEQNVNGFNTLGENIADNGGVRQAY
 KAYLKWMAEGGKDQQLPGLDLTHEQLFFINYAQVWCGSYRPEFAIQSIKTDVHSPKRYR
 LGSLQNLAAAFADTFHCARGTPMHPKERCRVW

Table 3: IGS5-DNA-1 ("IGS5DNA1") of SEQ ID NO: 3

5' -
 ATGGGGAAGTCCGAAGGCCCCGTGGGGATGGTGGAGAGCGCTGGCCGTGCAGGGCAGAAG
 CGCCCGGGGTTCCTGGAGGGGGGGCTGCTGCTGCTGCTGCTGCTGGTGACCGCTGCCCTG
 GTGGCCTTGGGTGTCCTCTACGCCGACCGCAGAGGGAAGCAGCTGCCACGCCTTGCTAGC
 CGGCTGTGCTTCTTACAGGAGGAGAGGACCTTTGTAAAACGAAAACCCCGAGGGATCCCA
 GAGGCCCCAAGAGGTGAGCGAGGTCTGCACCACCCCTGGCTGCGTGATAGCAGCTGCCAGG
 ATCCTCCAGAACATGGACCCGACCACGGAACCGTGTGACGACTTCTACCAGTTTGCATGC
 GGAGGCTGGCTGCGGCGCCACGTGATCCCTGAGACCAACTCAAGATACAGCATCTTTGAC
 GTCTCCGCGACGAGCTGGAGGTATCCTCAAAGCGGTGCTGGAGAATTCGACTGCCAAG
 GACCGGCCGGCTGTGGAGAAGGCCAGGACGCTGTACCGCTCCTGCATGAACCAGAGTGTG
 ATAGAGAAGCGAGGCTCTCAGCCCCTGCTGGACATCTTGGAGGTGGTGGGAGGCTGGCCG

GTGGCGATGGACAGGTGGAACGAGACCGTAGGACTCGAGTGGGAGCTGGAGCGGCAGCTG
GCGCTGATGAACTCACAGTTCAACAGGCGCGTCCTCATCGACCTCTTCATCTGGAACGAC
GACCAGAACTCCAGCCGGCACATCATCTACATAGACCAGCCACCTTGGGCATGCCCTCC
CGAGAGTACTACTTCAACGGCGGCAGCAACCGGAAGGTGCGGGAAGCCTACCTGCAGTTC
ATGGTGTCACTGGCCACGTTGCTGCGGGAGGATGCAAACCTGCCCAGGGACAGCTGCCTG
GTGCAGGAGGACATGATGCAGGTGCTGGAGCTGGAGACACAGCTGGCCAAGGCCACGGTA
CCCCAGGAGGAGAGACACGACGTCATCGCCTTGTACCACCGGATGGGACTGGAGGAGCTG
CAAAGCCAGTTTGGCCTGAAGGGATTTAACTGGACTCTGTTCATACAAACTGTGCTATCC
TCTGTCAAAATCAAGCTGCTGCCAGATGAGGAAGTGGTGGTCTATGGCATCCCCTACCTG
CAGAACCTTGAAAACATCATCGACACCTACTCAGCCAGGACCATAACAGAACTACCTGGTC
TGGCGCCTGGTGCTGGACCGCATTTGGTAGCCTAAGCCAGAGATTCAAGGACACACGAGTG
AACTACCGCAAGGCGCTGTTTGGCACAATGGTGGAGGAGGTGCGCTGGCGTGAATGTGTG
GGCTACGTCAACAGCAACATGGAGAACGCCGTGGGCTCCCTCTACGTCAGGGAGGCGTTC
CCTGGAGACAGCAAGAGCATGGTCAGAGAACTCATTGACAAGGTGCGGACAGTGTTTGTG
GAGACGCTGGACGAGCTGGGCTGGATGGACGAGGAGTCCAAGAAGAAGGCGCAGGAGAAG
GCCATGAGCATCCGGGAGCAGATCGGGCACCTGACTACATCCTGGAGGAGATGAACAGG
CGCCTGGACGAGGAGTACTCCAATCTGAACTTCTCAGAGGACCTGTACTTTGAGAACAGT
CTGCAGAACCTCAAGGTGGGCGCCAGCGGAGCCTCAGGAAGCTTCGGGAAAAGGTGGAC
CCAAATCTCTGGATCATCGGGGCGGCGGTGGTCAATGCGTTCTACTCCCCAAACCGAAAC
CAGATTGTATTCCCTGCCGGGATCCTCCAGCCCCCTTCTTCAGCAAGGAGCAGCCACAG
GCCTTGAACTTTGGAGGCATTGGGATGGTGATCGGGCACGAGATCACGCACGGCTTTGAC
GACAATGGCCGGAACCTTCGACAAGAATGGCAACATGATGGATTGGTGGAGTAACTTCTCC
ACCCAGCACTTCCGGGAGCAGTCAGAGTGCATGATCTACCAGTACGGCAACTACTCCTGG
GACCTGGCAGACGAACAGAACGTGAACGGATTCAACACCCTTGGGGAAAACATTGCTGAC
AACGGAGGGGTGCGGCAAGCCTATAAGGCCTACCTCAAGTGGATGGCAGAGGGTGGCAAG
GACCAGCAGCTGCCCCGGCCTGGATCTCACCATGAGCAGCTCTTCTTCATCAACTACGCC
CAGGTGTGGTGCGGGTCTTACCGGCCCCGAGTTCGCCATCCAATCCATCAAGACAGACGTC
CACAGTCCCCCTGAAGTACAGGGTACTGGGGTCTGCTGCAGAACCTGGCCGCCTTCGCAGAC
ACGTTCCACTGTGCCCCGGGGCACCCCCATGCACCCCAAGGAGCGATGCCGCGTGTGGTAG

Table 4: IGS5-protein-1 ("IGS5PROT1") of SEQ ID NO:4

MGKSEGPVGMVESAGRAGQKRPGFLEGGLLLLLLLVTAALVALGVLYADRRGKQLPRLAS
RLCFLQEERTFVKRKPRGIPEAQEVSEVCTTPGCVIAAARILQNMDPTTEPCDDFYQFAC
GGWLRRHVI PETNSRYSIFDVL RDELEVILKAVLENSTAKDRPAVEKARTLYRSCMNQSV
IEKRGSQPLLDILEVVGWPVAMDRWNETVGLEWELERQLALMNSQFNRRVLIDLFIWND
DQNSSRHIIYIDQPTLGMP SREYYFN GGSNRK VREAYLQFMVSVATLLREDANLPRDSCL
VQEDMMQVLELETQLAKATVPQEERHDVIALYHRMGLEELQSQFGLKGFNWTLFIQT VLS
SVKIKLLPDEEVVYGIPYLQNL ENIIDTYSARTIQNYLVWRLVLD RIGSLSQRFKDTRV
NYRKALFGTMVEEVRWRECVGYVNSNMENAVGSLYVREAFP GDSKSMVRELIDKVRTV FV
ETLDELGWMDEESKKKAQEKAMSIREQIGHDPDYILEEMNRRLDEEYSNLNFS EDLYFENS
LQNLKVG AQRSLRKLREKVDPNLWIIGA AVVNAFYSPNRNQIVFPAGILQPPFFSKEQPQ
ALNFGGIGMVIGHEITHGFDDNGRNFDKNGNMMDWWSNFS TQHFREQSECMIYQYGNYSW
DLADEQNVNGFNTLGENIADNGGVRQAYKAYLKWMAEGGKDQQLPGLDLTHEQLFFINYA
QVWCGSYRPEFAIQSIKTDVHSPLKYRVLGSLQNLAAAFADTFHCARGTPMHPKERCRVW

Table 5: IGS5-DNA-2 ("IGS5DNA2") of SEQ ID NO:5

5' -

ATGGGGAAGTCCGAAGGCCAGTGGGGATGGTGGAGAGCGCCGGCCGTGCAGGGCAGAAG
CGCCCGGGGTTCTTGAGGGGGGGCTGCTGCTGCTGCTGCTGCTGGTGACCGCTGCCCTG
GTGGCCTTGGGTGTCTCTACGCCGACCGCAGAGGGATCCCAGAGGCCAAGAGGTGAGC
GAGGTCTGCACCACCCCCTGGCTGCGTGATAGCAGCTGCCAGGATCCTCCAGAACATGGAC
CCGACCACGGAACCGTGTGACGACTTCTACCAGTTTGCATGCGGAGGCTGGCTGCGGCGC
CACGTGATCCCTGAGACCAACTCAAGATACAGCATCTTTGACGTCTCCGCGACGAGCTG
GAGGTCATCCTCAAAGCGGTGCTGGAGAATTCGACTGCCAAGGACCGGCCGGCTGTGGAG
AAGGCCAGGACGCTGTACCGCTCCTGCATGAACCAGAGTGTGATAGAGAAGCGAGGCTCT
CAGCCCCTGCTGGACATCTTGGAGGTGGTGGGAGGCTGGCCGGTGGCGATGGACAGGTGG
AACGAGACCGTAGGACTCGAGTGGGAGCTGGAGCGGCAGCTGGCGCTGATGAACTCACAG
TTCAACAGGCGCGTCTCATCGACCTCTTCATCTGGAACGACGACCAGAACTCCAGCCGG
CACATCATCTACATAGACCAGCCACCTTGGGCATGCCCTCCCGAGAGTACTACTTCAAC
GGCGGCAGCAACCGGAAGGTGCGGGAAGCCTACCTGCAGTTCATGGTGTCA GTGGCCACG
TTGCTGCGGGAGGATGCAAACCTGCCAGGGACAGCTGCCTGGTGCAGGAGGACATGATG
CAGGTGCTGGAGCTGGAGACACAGCTGGCCAAGGCCACGGTACCCCAGGAGGAGAGACAC

GACGTCATCGCCTTGTACCACCGGATGGGACTGGAGGAGCTGCAAAGCCAGTTTGGCCTG
AAGGGATTTAACTGGACTCTGTTCATACAAACTGTGCTATCCTCTGTCAAAATCAAGCTG
CTGCCAGATGAGGAAGTGGTGGTCTATGGCATCCCCCTACCTGCAGAACCTTGAAAACATC
ATCGACACCTACTCAGCCAGGACCATACAGAACTACCTGGTCTGGCGCCTGGTGCTGGAC
CGCATTGGTAGCCTAAGCCAGAGATTCAAGGACACACGAGTGAACTACCGCAAGGCGCTG
TTTGGCACAAATGGTGGAGGAGGTGCGCTGGCGTGAATGTGTGGGCTACGTCAACAGCAAC
ATGGAGAACGCCGTGGGCTCCCTCTACGTCAAGGAGGCGTTCCCTGGAGACAGCAAGAGC
ATGGTCAGAGAACTCATTGACAAGGTGCGGACAGTGTTTGTGGAGACGCTGGACGAGCTG
GGCTGGATGGACGAGGAGTCCAAGAAGAAGGCGCAGGAGAAGGCCATGAGCATCCGGGAG
CAGATCGGGCACCCCTGACTACATCCTGGAGGAGATGAACAGGCGCCTGGACGAGGAGTAC
TCCAATCTGAACTTCTCAGAGGACCTGTACTTTGAGAACAGTCTGCAGAACCTCAAGGTG
GGCGCCAGCGGAGCCTCAGGAAGCTTCGGGAAAAGGTGGACCCAAATCTCTGGATCATC
GGGGCGGCGGTGGTCAATGCGTTCTACTCCCCAAACCGAAACCAGATTGTATTCCTGCC
GGGATCCTCCAGCCCCCTTCTTCAGCAAGGAGCAGCCACAGGCCTTGAACCTTGGAGGC
ATTGGGATGGTGATCGGGACGAGATCAGCACGGCTTTGACGACAATGGCCGGAACCTC
GACAAGAATGGCAACATGATGGATTGGTGGAGTAACTTCTCCACCCAGCACTTCCGGGAG
CAGTCAGAGTGCATGATCTACCAGTACGGCAACTACTCCTGGGACCTGGCAGACGAACAG
AACGTGAACGGATTCAACACCCTTGGGGAAAACATTGCTGACAACGGAGGGGTGCGGCAA
GCCTATAAGGCCTACCTCAAGTGGATGGCAGAGGGTGGCAAGGACCAGCAGCTGCCCGGC
CTGGATCTCACCCTATGAGCAGCTCTTCTTCATCAACTACGCCCAGGTGTGGTGCGGGTCC
TACCGGCCCCGAGTTCGCCATCCAATCCATCAAGACAGACGTCCACAGTCCCCCTGAAGTAC
AGGGTACTGGGGTCGCTGCAGAACCTGGCCGCCTTCGCAGACACGTTCCTACTGTGCCCGG
GGCACCCCCATGCACCCCAAGGAGCGATGCCGCGTGTGGTAG - 3'

Table 6: IGS5-protein-2 ("IGS5PROT2") of SEQ ID NO:6

MGKSEGPVGMVESAGRAGQKRPGFLEGLLLLLLLLLVTAALVALGVLYADRRGIPEAQEVS
EVCTTPGCVIAAARILQNMDPTTEPCDDFYQFACGGWLR RHVIPETNSRYSIFDVL RDEL
EVILKAVLENSTAKDRPAVEKARTLYRSCMNQSVIEKRG SQPLLDILEVVGGWPVAMDRW
NETVGLEWELERQLALMNSQFNRRVLIDLFIWNDQNSSRHI IYIDQPTLGMP SREYYFN
GGSNRKVREAYLQFMVSVATLLREDANLPRDSCLVQEDMMQVLELETQLAKATVPQEERH
DVIALYHRMGLEELQSQFGLKGFNWT LFIQTVLSSVKIKLLPDEEVVVG IPIYLQNLNI
IDTYSARTIQNYLVWRLVLDRI GSLSQRFKDRVNYRKALFGTMVEEVRWRECVGYVNSN
MENAVGSLYVREAPGDSKSMVRELIDKVRTVFVETLDELGWMDEESKKKAQEKAMSIRE

QIGHPDYILEEMNRRRLDEEYSNLNFSEDLYFENSLQNLKVGARSRLKREKVDPNLWII
GAAVVNAFYSPNRNQIVFPAGILQPPFFSKEQPQALNFGGIGMVGHEITHGFDDNGRNF
DKNGNMMDWWSNFSSTQHFREQSECMYQYGNYSWDLADEQNVNGFNTLGENIADNGGVRQ
AYKAYLKWMAEGGKDQQLPGLDLTHEQLFFINYAQVWCGSYRPEFAIQSIKTDVHSPLKY
RVLGSLQNLAAFADTFHCARGTPMHPKERCRVW

DETAILED DESCRIPTION OF THE INVENTION

Definitions

5 The following definitions are provided to facilitate understanding of certain terms used frequently herein.

 "IGS5" refers, among others, to a polypeptide comprising the amino acid sequence set forth in one of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, or respective
10 variants thereof. Thus "IGS5" particularly includes IGS5PROT, IGS5PROT1 and IGS5PROT2.

 "Enzyme Activity" or "Biological Activity" refers to the metabolic or physiologic function of said IGS5 including similar activities or improved activities or these activities
15 with decreased undesirable side effects. Also included are antigenic and immunogenic activities of said IGS5.

 "IGS5-gene" refers to a polynucleotide comprising the nucleotide sequence set forth in one of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, or respective variants, e.g.
20 allelic variants, thereof and/or their complements.

 "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of a Fab or other immunoglobulin expression library.
25

 "Isolated" means altered "by the hand of man" from the natural state and/or separated from the natural environment. Thus, if an "isolated" composition or substance that occurs in nature has been "isolated", it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally
30 present in a living animal is not "isolated", but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, biotinylation, covalent attachment of flavin,

covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol; cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination (see, for instance, "Proteins - Structure and Molecular Properties", 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993; Wold, F., "Post-translational Protein Modifications: Perspectives and Prospects", pp. 1-12 in "Post-translational Covalent Modification of Proteins", B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", Meth. Enzymol. (1990) 182:626-646; and Rattan et al., "Protein Synthesis: Post-translational Modifications and Aging", Ann. NY Acad. Sci. (1992) 663:48-62).

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, and deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity", as known as a measure of identity in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences, e.g. in generally by alignment of the sequences so that the highest order match is obtained. Thus "Identity" and or the alternative wording "Similarity" has an art-recognized meaning and can be readily calculated by known methods, including but not limited to those described in "Computational Molecular Biology", Lesk, A.M., Ed., Oxford University Press, New York, 1988; "Biocomputing: Informatics and Genome Projects", Smith, D.W., Ed., Academic Press, New York, 1993; "Computer Analysis of Sequence Data", Part I, Griffin, A.M., and Griffin, H.G., Eds., Humana Press, New Jersey, 1994; "Sequence Analysis in Molecular Biology", von Heinje, G., Academic Press, 1987; "Sequence Analysis Primer", Gribskov, M. and Devereux, J., Eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., J. Molec. Biol. 215: 403-410 (1990)). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990)). The well known Smith Waterman algorithm may also be used to determine identity. A publicly available program useful to determine identity or similarity of polypeptide sequences or polynucleotide sequence, respectively, is known as the "gap" program from Genetics Computer Group, Madison WI, which is usually run with the default parameters for comparisons (along with no penalty for end gaps). The preferred (i.e. default) parameters for polypeptide sequence comparison include the following: Algorithm as described by Needleman and Wunsch, J. Mol. Biol. 48: 443-453 (1970); Comparison Matrix BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992); Gap Penalty: 12; Gap Length Penalty: 14. The preferred (i.e. default) parameters for polynucleotide sequence comparison include the following: Algorithm as described by Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970); Comparison Matrix: matches =

+10, mismatch = 0; Gap Penalty: 50; Gap Length Penalty: 3. The word "homology" may substitute for the word "identity".

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence, for example to a reference nucleotide sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the respective reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence, or in a number of nucleotides of up to 5% of the total nucleotides in the reference sequence there may be a combination of deletion, insertion and substitution. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

20

Similarly, by a polypeptide having an amino acid sequence having at least, for example 95% "identity" to a reference amino acid sequence, for example to a reference amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the respective reference amino acid. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino- or carboxy-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among

25

30

residues in the reference sequence or in one or more contiguous groups within the reference sequence.

"Homolog" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a subject sequence. Such relatedness may be quantified by determining the degree of identity and/or similarity between the sequences being compared as herein described. Falling within this generic term are the terms "ortholog", meaning a polynucleotide or polypeptide that is the functional equivalent of a polynucleotide or polypeptide in another species, and "paralog" meaning a functionally similar sequence when considered within the same species. Hence, in humans for example, within the family of endothelin converting enzymes ECE-1 is a paralog of the other members, e.g. of ECE-2.

"Fusion protein" refers to a protein encoded by two, often unrelated, fused genes or fragments thereof. This term may be illustrated for example by fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, employing an immunoglobulin Fc region as a part of a fusion protein is advantageous for use in therapy and diagnosis resulting in, for example, improved pharmacokinetic properties (see, e.g., EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified.

Polypeptides of the Invention

The present invention relates to IGS5 polypeptides (or IGS5 enzymes, e.g. to IGS5PROT, IGS5PROT1 or IGS5PROT2, respectively), in particular to human IGS5 polypeptides (or human IGS5 enzymes), and also to IGS5 polypeptide fragments comprising a substantial portion of said entire IGS5 polypeptide. Thus, in a first aspect, the IGS5 polypeptides of the present invention include isolated polypeptides, in particular isolated human species polypeptides, comprising an amino acid sequence which has at least 70% identity, preferably at least 80% and in particular at least 85 % identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to one of that selected from the group of SEQ ID NO:2, SEQ ID NO:4 SEQ and SEQ ID NO:6, over the entire length of the respective SEQ ID NO:2, SEQ ID NO:4 SEQ and SEQ ID NO:6. Such polypeptides include those comprising one of the

amino acid sequences selected from the group of SEQ ID NO:2, SEQ ID NO:4 SEQ and ID NO:6,.

In a second aspect, the IGS5 polypeptides of the present invention include
5 isolated polypeptides, in particular isolated human IGS5 polypeptides, having an amino acid sequence of at least 70% identity, preferably at least 80% and in particular at least 85 % identity, more preferably at least 90% identity, yet more preferably at least 95% identity, most preferably at least 97-99% identity, to one of the amino acid sequences selected from the group of SEQ ID NO:2, SEQ ID NO:4 SEQ and ID NO:6, over the entire
10 length of the respective SEQ ID NO:2, SEQ ID NO:4 SEQ and ID NO:6. Such polypeptides include the IGS5 polypeptide of SEQ ID NO:2, of SEQ ID NO:4 and SEQ ID NO:6, respectively.

Further polypeptides of the present invention include isolated IGS5 polypeptides
15 comprising the sequence contained in one of SEQ ID NO:2, SEQ ID NO:4 SEQ and ID NO:6, and which in particular are human species polypeptides.

Polypeptides of the present invention are members of the metalloprotease family of polypeptides. They are of interest because several dysfunctions, disorders or diseases
20 have been identified where metalloproteases play a critical role in the pathology of the disease. Examples of the diseases, in context of which the use of the polypeptides and polynucleotides of the present invention is thought to be useful, include amongst others: CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD),
25 phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles dela Tourett's syndrome, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyper-
30 activity disorder (ADHD); cardiovascular diseases including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g.

renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophageal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post-operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus. The Polypeptides of the present invention are in particular of interest in the context of cardiovascular diseases. Furthermore, the IGS5 polypeptides of the invention are also of interest for identifying stimulators or inhibitors of these polypeptides, for providing diagnostic assays for detecting diseases associated with inappropriate IGS5 activity or levels, and for treating conditions associated with IGS5 imbalance with compounds identified to be stimulators or inhibitors. Hence, the IGS5 polypeptides of the invention may be used for designing or screening for selective stimulators or inhibitors, and thus can lead to the development of new drugs. The properties of the IGS5 polypeptides, in particular of the human species IGS5 polypeptides, of the present invention are hereinafter referred to as "IGS5 activity" or "IGS5 polypeptide activity" or "biological activity of IGS5". Also included amongst these activities are antigenic and immunogenic activities of said IGS5 polypeptides, in particular the antigenic and immunogenic activities of one of the polypeptides selected from the group of SEQ ID NO:2, SEQ ID NO:4 SEQ and ID NO:6. Preferably, a polypeptide of the present invention exhibits at least one biological activity of IGS5, preferably of human IGS5.

25

The IGS5 polypeptides of the present invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

30

The present invention also includes variants of the aforementioned polypeptides, that is polypeptides that vary from the referents by conservative amino acid substitutions,

whereby a residue is substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr, among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

The present invention furthermore pertains to fragments of the IGS5 polypeptides, in particular to IGS5 polypeptide fragments comprising a substantial portion of the entire IGS5 polypeptide. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned IGS5 polypeptides. As with IGS5 polypeptides, fragments may be "free-standing", or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region.

Preferred fragments include, for example, truncation polypeptides having the amine acid sequence of IGS5 polypeptides, except for deletion of a continuous series of residues that includes the amine terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amine terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are these that mediate enzyme activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

With regard to the variant of the invention pertaining to polypeptide fragments comprising a substantial portion of the entire IGS5 polypeptide as shown in one of SEQ ID NO:2, SEQ ID NO:4 SEQ and ID NO:6, the term "substantial" has the meaning that the fragment of the IGS5 polypeptide has in particular a size of at least about 50 amino acids, preferably a size of at least about 100 amino acids, more preferably a size of at least about 200 amino acids, most preferably a size of at least about 300 amino acids. In this context "about" includes the particularly recited sizes larger or smaller by several, 5, 4, 3,

2 or 1 amino acids. The IGS5 polypeptide fragments according to the invention preferably show at least to some extent at least one of the properties which are characteristic for the IGS5 polypeptides themselves.

5 With regard to the IGS5 polypeptides of the present invention it was found that they may be involved in the metabolism of biologically active peptides. In particular it was found that these IGS5 polypeptides are metalloprotease type enzymes which may act on a variety of vasoactive peptides. Vasoactive peptides known in the state of the art are e.g. such like atrial natriuretic peptide (ANP), bradykinin, big endothelin (Big ET-1), endothelin
10 (ET-1), substance P, and angiotensin-1. In the context of the present invention it was found that the IGS5 ectodomain, which is a novel human metalloprotease, hydrolyzes e.g. in vitro a variety of said vasoactive peptides including Big ET-1, ET-1, ANP and bradykinin.

15 Furthermore, the IGS5 metalloprotease type enzymes of the present invention may be inhibited by reference compounds that are used to determine the inhibition properties with regard to enzymes having ECE/NEP-characteristics, e.g. inhibition by compounds such like phosphoramidon. No inhibition of IGS5 is observed for reference compounds that specifically inhibit NEP, e.g. no inhibition of IGS5 by compounds such as
20 thiorphan. Nor any inhibition of IGS5 is observed for reference compounds that specifically inhibit ECE, e.g. no inhibition of IGS5 by compounds such as the selective ECE inhibitor CGS-35066 (De Lombart et al., J. Med. Chem. 2000, Feb. 10; 43(3):488-504). The inhibition data of these reference compounds with regard to the inhibition of the IGS5 metalloprotease type enzymes of the present invention are further described in the
25 experimental part below, in particular in Example 7.

Polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by
30 a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

In another aspect, the present invention relates to IGS5 polynucleotides (e.g. to
35 IGS5DNA, IGS5DNA1 or IGS5DNA2, respectively), in particular to human IGS5

polynucleotides. Such polynucleotides include isolated polynucleotides, preferably isolated human species polynucleotides, comprising a nucleotide sequence encoding a polypeptide which has at least 70% identity, preferably at least 80% and in particular at least 85 % identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to one of the amino acid sequences selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, over the entire length of the respective SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6. In this regard, polynucleotides encoding polypeptides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identity are more highly preferred, and those with at least 99%, in particular 99.9%, identity are most highly preferred. Such polynucleotides include polynucleotides comprising the nucleotide sequence contained in one of the SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, encoding the respective polypeptide of SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6.

In a variant of this aspect, the polynucleotides of the present invention include isolated polynucleotides, in particular isolated human polynucleotides, comprising a nucleotide sequence that has at least 70% identity, preferably at least 80% and in particular at least 85 % identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to a nucleotide sequence encoding one of the polypeptides selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, over the entire coding region. In this regard, polynucleotides which have at least 97% identity are highly preferred, whilst those with at least 98-99% identity are more highly preferred, and those with at least 99%, in particular 99.9%, identity are most highly preferred.

Further polynucleotides of the present invention include isolated polynucleotides, in particular isolated human polynucleotides, comprising a nucleotide sequence which has at least 70% identity, preferably at least 80% and in particular at least 85 % identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to one of the nucleotide sequences selected from the group of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO: 5, over the entire length of the respective SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5. Particularly, polynucleotides of the present invention include isolated polynucleotides having a nucleotide sequence of at least 70% identity, preferably at least 80% and in particular at least 85 % identity, more preferably at least 90% identity, yet more preferably at least 95% identity, to the respective reference nucleotide sequence

over the entire length of the reference nucleotide sequence. In this regard, polynucleotides which comprise or have a nucleotide sequence of at least 97% identity to one of the nucleotide sequences selected from the group of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5 are highly preferred, whilst those with at least 98-99% identity, are more highly preferred, and those with at least 99%, in particular 99.9%, identity are most highly preferred. Such polynucleotides include a polynucleotides comprising one of the polynucleotides of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, as well as the polynucleotides of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5 themselves, which in particular are human species polynucleotides.

The invention also provides polynucleotides which are complementary to all the above described polynucleotides.

The nucleotide sequence of SEQ ID NO:1 (designated "IGS5DNA") is a cDNA sequence from human origin (*Homo sapiens*) with a length of 2076 nucleotides and comprises a polypeptide encoding sequence (from nucleotide no. 1 to no. 2073) encoding a polypeptide of 691 amino acids, the polypeptide of SEQ ID NO:2 (designated "IGS5PROT"). The nucleotide sequence encoding the polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:1 or it may be a sequence other than the one contained in SEQ ID NO:1, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

The nucleotide sequence of SEQ ID NO:3 (designated "IGS5DNA1") is a cDNA sequence from human origin (*Homo sapiens*) with a length of 2340 nucleotides (including the stop codon tag) and comprises a polypeptide encoding sequence (from nucleotide no. 1 to no. 2337) encoding a polypeptide of 779 amino acids, the polypeptide of SEQ ID NO:4 (designated "IGS5PROT1"). The nucleotide sequence encoding the polypeptide of SEQ ID NO:4 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:3 or it may be a sequence other than the one contained in SEQ ID NO:3, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:4.

The nucleotide sequence of SEQ ID NO:5 (designated "IGS5DNA2") is a cDNA sequence from human origin (*Homo sapiens*) with a length of 2262 nucleotides (including the stop codon tag) and comprises a polypeptide encoding sequence (from nucleotide no. 1 to no. 2259) encoding a polypeptide of 753 amino acids, the polypeptide of SEQ ID NO:6 (designated "IGS5PROT2"). The nucleotide sequence encoding the polypeptide of SEQ ID NO:6 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:5 or it may be a sequence other than the one contained in SEQ ID NO:5, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:6.

The characteristics of the type of polypeptides encoded by the polynucleotides of the invention are described in more detail below.

Biological and Pharmacological Features of Metalloproteases

The polypeptides of the present invention, and in particular those being human species polypeptides, are structurally and functionally related to other proteins of the metalloprotease family, e.g. showing homology and/or structural similarity with metalloproteases or related enzymes, such as e.g. MMPs, ACE, ECE or NEP, respectively. Thus, for example, the polypeptide of the SEQ ID NO:2 is structurally and functionally related to other proteins of the metalloprotease family, having homology and/or structural similarity with enzymes such as NEP or ECE (e.g. ECE-1), and in particular with NEP. Thus, preferred polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides. Furthermore, preferred polypeptides and polynucleotides of the present invention have at least one IGS5 activity.

The general features of metalloproteases and their activities, in particular with regard to the present invention, are already described above. For further understanding of the nature and characteristics of the polypeptides and polynucleotides of the present invention, in particular the function of these polypeptides and polynucleotides, some more specific features of each of the enzymes like MMPs, ACE, ECE or NEP, respectively, are summarized as follows.

Matrix metalloproteases (MMPs), also designated matrixins, are a family of zinc metalloproteases that function in the turnover of components of the extracellular matrix. To date, several members of the matrixin family have been identified in humans. MMPs are synthesized and secreted from a number of cell types such as fibroblasts, epithelial cells, phagocytes, lymphocytes and cancer cells. MMPs are synthesized as pre-pro-enzymes which are destined to be secreted as proenzymes from all producing cells except neutrophils. Under physiological conditions these enzymes play central roles in morphogenesis, tissue remodelling and resorption. In excess, they participate in the destruction of the extracellular matrix associated with many connective tissue diseases such as in arthritis, periodontitis, glomerulonephritis, and with cancer cell invasion and metastasis. Thus, the MMPs play a central role, for example, in the normal embryo genesis and tissue remodelling and in many diseases such as arthritis, cancer, periodontitis, glomerulonephritis, encephalomyelitis, atherosclerosis and tissue ulceration. The importance of the matrixins in both physiological and pathological catabolism of connective tissue matrix has been emphasized, because little MMP activity can be detected in normal steady-state tissues, but the synthesis of many MMPs is transcriptionally regulated by inflammatory cytokines, hormones, growth factors and on cellular transformation. The biological activities of MMPs are further controlled extracellularly during steps in their activation from inactive precursors (proMMPs), as well as through interaction with the extracellular substratum and endogenous inhibitors. The MMPs are an important class of zinc-dependent metalloproteases involved in degradation and remodeling of the extracellular matrix. Inhibitors of these enzymes have therapeutic potential in e.g. cancer, arthritis, osteoporosis and Alzheimer's disease, and several of these inhibitors are under clinical evaluation.

Angiotensin I Converting Enzyme (ACE; peptidyl dipeptidase A; EC 3.4.15.1) is a member of the angiotensin converting enzyme family of zinc metalloproteases. ACE is primarily expressed at the surface of endothelial, epithelial and neuroepithelial cells (somatic ACE) as an ectoenzyme, meaning that it is anchored to the plasma membrane with the bulk of its mass, including its catalytic site/s, facing the extracellular milieu. ACE is found in the plasma membrane of vascular endothelial cells, with high levels found at the vascular endothelial surface of the lung such that the active sites of ACE are posed to metabolize circulating substrates. In addition to the endothelial location of ACE, the enzyme is also expressed in the brush borders of absorptive epithelia of the small

intestine and the kidney proximal convoluted tubule. ACE is also found in mononuclear cells, such as monocytes after macrophage differentiation and T-lymphocytes, and in fibroblasts. In vitro autoradiography, employing radiolabelled specific ACE inhibitors, and immunohistochemical studies have mapped the principal locations of ACE in the brain.

5 ACE was found primarily in the choroid plexus, which may be the source of ACE in cerebrospinal fluid, ependyma, subfornical organ, basal ganglia (caudate-putamen and globus pallidus), substantia nigra and pituitary. A soluble form of ACE has been detected in many biological fluids such as serum, seminal fluid, amniotic fluid and cerebrospinal fluid. The soluble form of ACE appears to be derived from the membrane-bound form of
10 the enzyme in endothelial cells. A main physiological activity of ACE is that it cleaves the C-terminal dipeptide from angiotensin I to produce the potent vasopressor peptide angiotensin II and inactivates the vasodilatory peptide bradykinin by the sequential removal of two C-terminal dipeptides. As a consequence of the involvement of ACE in the metabolism of these two vasoactive peptides angiotensin II and bradykinin, ACE has
15 become a crucial molecular target in the treatment of hypertension and congestive heart failure. This has led to the development of highly potent and specific ACE inhibitors which have become clinically important and widespread as orally active drugs to control these conditions of hypertension and congestive heart failure. Whilst the metabolism of vasoactive peptides remains the best known physiological function of ACE, the enzyme
20 has been also implicated in a range of other physiological processes unrelated to blood pressure regulation such as immunity, reproduction and neuropeptide metabolism due to the localization of ACE and/or the in vitro cleavage of a range of biologically active peptides.

25 Neutral Endopeptidase (NEP, neprilysin, EC 3.4.24.11) is a zinc metalloprotease and classified as a member of the neprilysin family. NEP was first isolated from the brush border membranes of rabbit kidney. Later, an NEP-like enzyme was identified in rat brain as being involved in the degradation of the opioid peptides, enkephalins. The cloning of the ectoenzyme NEP and subsequent site-directed mutagenesis experiments have shown
30 that, as well as having a similar specificity to thermolysin, it also has a similar active site organization. NEP also shows a thermolysin-like specificity for cleaving peptides on the N-terminal side of hydrophobic residues. With regard to the general distribution of NEP it has been determined in the brain and spinal cord, and lesion and electron microscopic studies generally support a predominantly neuronal localization of NEP, although the

enzyme could be present on oligodendrocytes surrounding the fibers of the striato-pallidal and striato-nigral pathways and on Schwann cells in the peripheral nervous system. NEP does not appear to be concentrated on specific membrane interfaces such as the synapse, but is rather uniformly distributed on the surface of neuronal perikarya and dendrites. In the periphery, NEP is particularly abundant in the brush border membranes of the kidney and intestine, the lymph nodes and the placenta, and is found in lower concentrations in many other tissues including the vascular wall of the aorta. By finding that the common acute lymphoblastic leukemia antigen was NEP, it was also shown in the state of the art that the enzyme is transiently present at the surface of lymphohaemopoietic cells and elevated levels are found on mature lymphocytes in certain disease states. The clinical interest in NEP, in particular the interest in NEP inhibitors as potential clinical agents derives from the actions of NEP, in conjunction with another zinc metalloprotease, the aminopeptidase N (APN, membrane alanyl aminopeptidase, EC 3.4.11.2), in degrading the enkephalins and also from its role in degrading atrial natriuretic peptide (ANP). For example, it is known that dual inhibitors of NEP and angiotensin converting enzyme (ACE) are potent antihypertensives, resulting from simultaneously increasing the circulating levels of atrial natriuretic peptide, due to NEP inhibition, and decreasing the circulating levels of angiotensin II, due to ACE inhibition. Further interest in the clinical potential of NEP inhibitors came when the peripheral enzyme was shown to degrade the circulating natriuretic and diuretic peptide, atrial natriuretic peptide. NEP inhibitors were therefore investigated for their antihypertensive properties. From a further example it is known that inhibition of enkephalin metabolism by the synthetic NEP inhibitor, thiorphan, gave naloxone-reversible antinociceptive responses in mice. This opened the possibility that, by increasing the levels of endogenous opioids in the regions of their target receptors, an analgesia could be obtained relatively free of the side-effects of morphine or other classical opiate drugs. It was realized that in order to achieve any significant effect, other enkephalin-metabolizing enzymes also had to be inhibited, in particular the aminopeptidase N (APN). Such dual NEP/APN inhibitors completely block enkephalin metabolism and have strong antinociceptive properties.

Endothelin Converting Enzyme (ECE) catalyses the final step in the biosynthesis of the potent vasoconstrictor peptide endothelin (ET). This involves cleavage of the Trp-Val bond in the inactive intermediate, big endothelin. ECE-1 is a zinc metalloprotease which is homologous with neutral endopeptidase (NEP; neprilysin; EC 3.4.24.11, see

above). Like NEP, ECE-1 is inhibited by the compound phosphoramidon and is a type II integral membrane protein. Unlike NEP, however, ECE-1 exists as a disulfide-linked dimer and is not inhibited by other NEP inhibitors such as thiorphan. Immunocytochemical studies indicate a predominant cell-surface location for ECE-1 where it exists as an ectoenzyme. ECE-1 is localized to endothelial cells and some secretory cells, e.g. β -cells in the pancreas, and in smooth muscle cells. Potent and selective inhibitors of ECE, or dual inhibitors of ECE and NEP, may have therapeutic applications in cardiovascular and renal medicine. Endothelin (ET) which is a 21 amino acid bicyclic peptide containing two intramolecular disulfide bonds, is one of the most potent vasoconstricting peptides identified to date and administration to animals results in a sustained increase in blood pressure emphasizing its potential role in cardiovascular regulation. The endogenous production of ET-1 in humans contributes to the maintenance of basal vascular tone. The endothelin system and related enzymes like ECE therefore represent a likely candidate for the development of novel pharmaceutical agents. Thus, the clinical interest in ECE, in particular the interest in ECE inhibitors as potential clinical agents derives from the actions of ECE, in particular in the context of the biosynthesis of ET. Consequently, compounds showing a significant endothelin converting enzyme inhibitory activity are useful in treating and preventing various diseases which are induced or suspected to be induced by ET, such as for example, cardiovascular diseases including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; asthma; stroke, Alzheimer's disease; complication of diabetes mellitus; ulcer such as gastric ulcer; cancer such as lung cancer; endotoxin shock; sepsis; and the like.

The Polypeptides of the present invention are in particular of interest in the context of cardiovascular diseases.

Procedures for Obtaining Polynucleotides of the Present Invention

Polynucleotides of the present invention may be obtained, using standard cloning and screening techniques, from a cDNA library derived from mRNA in cells of human testis tissue, using the expressed sequence tag (EST) analysis (Adams, M.D., et al. Science (1991) 252:1651-1656; Adams, M.D. et al., Nature, (1992) 355:632-634; Adams,

M.D., et al., Nature (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques (e.g. F.M. Ausubel et al., 2000, Current Protocols in Molecular Biology).

5

When polynucleotides of the present invention are used for the recombinant production of polypeptides of the present invention, the polynucleotide may include the coding sequence for the mature polypeptide, by itself; or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et al., Proc Natl Acad Sci USA (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further embodiments of the present invention include polynucleotides encoding polypeptide variants which comprise one of the amino acid sequences selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, and in which several, for instance from 5 to 10, 1 to 5, 1 to 3, 1 to 2 or 1, amino acid residues are substituted, deleted or added, in any combination.

Polynucleotides which are identical or sufficiently identical to a nucleotide sequence contained in one of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, may be used as hybridization probes for cDNA and genomic DNA or as primers for a nucleic acid amplification (PCR) reaction, to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes (including genes encoding paralogs from human sources and orthologs and paralogs from species other than human) that have a high sequence similarity to one of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5. Typically these nucleotide sequences are at least 70% identical, preferably at least 80% and in particular at least 85 % identical, more preferably at least 90% identical, most preferably at least 95% identical to that of the

referent. The probes or primers will generally comprise at least 15 nucleotides, preferably, at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will have between 30 and 50 nucleotides. Particularly preferred primers will have between 20 and 25 nucleotides.

5

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than human, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of one of SEQ ID NO: 1, SEQ ID NO:3 or SEQ ID NO:5, or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to the skilled artisan. Preferred stringent hybridization conditions include overnight incubation at 42 °C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate (w/v), and 20 microgram/ml denatured, sheared salmon sperm DNA; followed by washing the filters in 0.1 x SSC at about 65 °C. Thus the present invention also includes polynucleotides obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of one of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, or a fragment thereof.

20

The skilled artisan will appreciate that, in many cases, an isolated cDNA sequence will be incomplete, in that the region coding for the polypeptide is cut short at the 5' end of the cDNA. This is a consequence of reverse transcriptase, an enzyme with inherently low "processivity" (a measure of the ability of the enzyme to remain attached to the template during the polymerisation reaction), failing to complete a DNA copy of the mRNA template during 1st strand cDNA synthesis.

25

There are several methods available and well known to those skilled in the art to obtain full-length cDNAs, or extend short cDNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman et al., PNAS USA 85, 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an "adaptor" sequence ligated onto

30

each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that
5 anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length cDNA constructed either by joining the product directly to the existing cDNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

10

Vectors, Host Cells, Expression

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression
15 systems which comprise a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

20

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., Basic Methods in
25 Molecular Biology (1986) and Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). Preferred such methods include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

30

Representative examples of appropriate hosts include bacterial cells, such as Streptococci, Staphylococci, E. coli, Streptomyces and Bacillus subtilis cells; fungal cells, such as yeast cells and Aspergillus cells; insect cells such as Drosophila S2 and

Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

5 A great variety of expression systems can be used, for instance, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from
10 plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector which is able to maintain, propagate or express a polynucleotide to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety
15 of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., Molecular Cloning, A Laboratory Manual (supra). Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be
20 heterologous signals, i.e. derived from a different species.

If a polypeptide of the present invention is to be expressed for use in screening assays, it is generally possible that the polypeptide be produced at the surface of the cell or alternatively in a soluble protein form. If the polypeptide is secreted into the medium,
25 the medium can be recovered in order to recover and purify the polypeptide. If produced intracellularly, the cells must first be lysed before the polypeptide is recovered. If the polypeptide is bound at the surface of the cell (membrane bound polypeptide), usually membrane fractions are prepared in order to accumulate the membrane bound polypeptide.

30

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography,

hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

5

Diagnostic Assays

This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of the gene characterized by one of the the polynucleotides selected from the group of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID
10 NO:5, which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered spatial or temporal expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques.

15

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion.
20 Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled IGS5 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in
25 electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (ee, e.g., Myers et al., Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton et al., Proc Natl Acad Sci USA (1985) 85: 4397-4401). In another embodiment, an array of oligo-
30 nucleotides probes comprising IGS5 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to the Diseases through detection of mutation in the IGS5 gene by the methods described. In addition, such diseases may be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of polypeptide or mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as a polypeptide of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radio-immuno-assays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

- (a) a polynucleotide of the present invention, preferably the nucleotide sequence of one of SEQ ID NO: 1, SEQ ID NO:3 or SEQ ID NO:5, or a fragment thereof;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a polypeptide of the present invention, preferably the polypeptide of one of SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof; or
- (d) an antibody to a polypeptide of the present invention, preferably to one of the polypeptides of SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6.

It will be appreciated that in any such kit, the component (a), (b), (c) or (d) may constitute a substantial component of said diagnostic kit. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, particularly amongst others a disease as indicated above in the context of the polypeptides of the present invention.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome localization. The sequence is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in corre-

lating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns
5 Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and un-
10 affected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Tissue Localization

15 The nucleotide sequences of the present invention are also valuable for tissue localization. Such techniques allow the determination of expression patterns of the IGS5 polypeptides in tissues by detection of the mRNAs that encode them. These techniques include in situ hybridization techniques and nucleotide amplification techniques, for example PCR. Such techniques are well known in the art. Results from these studies
20 provide an indication of the normal functions of the polypeptides in the organism. In addition, comparative studies of the normal expression pattern of IGS5 mRNAs with that of mRNAs encoded by a IGS5 gene provide valuable insights into the role of mutant IGS5 polypeptides, or that of inappropriate expression of normal IGS5 polypeptides, in disease. Such inappropriate expression may be of a temporal, spatial or simply quantitative nature.

25 The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them, can also be used as immunogens to produce antibodies immunospecific for polypeptides of the present invention. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than
30 their affinity for other related polypeptides in the prior art.

Antibodies

Antibodies generated against polypeptides of the present invention may be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to

an animal, preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, 77-96, Alan R. Liss, Inc., 1985). Techniques for the production of single chain antibodies, such as those described in U.S. Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against polypeptides of the present invention may also be employed to treat the diseases as indicated above, amongst others.

Fusion Proteins

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises administering to (for example by inoculation) the

mammal a polypeptide of the present invention, adequate to produce antibody and/or T cell immune response to protect said animal from the Diseases hereinbefore mentioned, amongst others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering a polypeptide of the present invention via a vector directing expression of the polynucleotide and coding for the polypeptide in vivo in order to induce such an immunological response to produce antibody to protect said animal from diseases.

A further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a polypeptide of the present invention wherein the composition comprises a polypeptide or polynucleotide of the present invention. Such immunological/vaccine formulations (compositions) may be either therapeutic immunological/vaccine formulations or prophylactic immunological/vaccine formulations. The vaccine formulation may further comprise a suitable carrier. Since a polypeptide may be broken down in the stomach, it is preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

30 **Screening Assays**

Polypeptides of the present invention are responsible for one or more biological functions, including one or more disease states, in particular the Diseases hereinbefore mentioned. It is therefore desirous to devise screening methods to identify compounds which stimulate or which inhibit the function of the polypeptide. Accordingly, in a further

aspect, the present invention provides for a method of screening compounds to identify those which stimulate or which inhibit the function of the polypeptide. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such stimulators or inhibitors so-identified may be
5 natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide; or may be structural or functional mimetics thereof (see Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991)).

The screening method may simply measure the influence of a candidate compound on the activity of the polypeptide, or on cells or membranes bearing the polypeptide. Alternatively, the screening method may involve competition with a competitor. Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the activity of the polypeptide or to the cells or membranes bearing the
15 polypeptide. Inhibition of polypeptide activity is generally assayed in the presence of a known substrate and the effect of the candidate compound is observed by altered activity, e.g. by testing whether the candidate compound results in inhibition or stimulation of the polypeptide. For example, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide of the present
20 invention, and a suitable substrate to form a mixture, measuring IGS5 activity in the mixture, and comparing the IGS5 activity of the mixture to a standard without candidate compound.

The polynucleotides, polypeptides and antibodies to the polypeptide of the present
25 invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production
30 of polypeptide from suitably manipulated cells or tissues.

Examples of potential polypeptide inhibitors include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, e.g., a fragment of the ligands,

substrates, receptors, enzymes, etc. ; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

5 Thus, in another aspect, the present invention relates to a screening kit for identifying in particular inhibitors, stimulators, ligands, receptors, substrates, enzymes, etc. for polypeptides of the present invention; or compounds which decrease or enhance the production of such polypeptides, which comprises:

- (a) a polypeptide of the present invention;
- 10 (b) a recombinant cell expressing a polypeptide of the present invention;
- (c) a cell membrane expressing a polypeptide of the present invention; or
- (d) an antibody to a polypeptide of the present invention; which polypeptide is preferably one of that of SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6.

15 It will be appreciated that in any such kit, the component (a), (b), (c) or (d) may constitute a substantial part of said kit.

 It will be readily appreciated by the skilled artisan that a polypeptide of the present invention may also be used in a method for the structure-based design of a stimulator or inhibitor of the polypeptide, by:

- (a) determining in the first instance the three-dimensional structure of the polypeptide;
- (b) deducing the three-dimensional structure for the likely reactive or binding site(s) of a stimulator or inhibitor;
- 25 (c) synthesizing candidate compounds that are predicted to bind to or react with the deduced binding or reactive site; and
- (d) testing whether the candidate compounds are indeed stimulators or inhibitors.

It will be further appreciated that this will normally be an iterative process.

30 **Prophylactic and Therapeutic Methods**

 In a further aspect, the present invention provides methods of treating abnormal conditions such as, for instance, those dysfunctions, disorders or diseases to be treated, hereinabove generally referred to as "the diseases" in the context of the polypeptides of

the present invention, related to either an excess of, or an under-expression of IGS5 polypeptide activity.

If the activity of the polypeptide is in excess, several approaches are available.

5 One approach comprises administering to a subject in need thereof an inhibitor compound as hereinabove described, optionally in combination with a pharmaceutically acceptable carrier, in an amount effective to inhibit the function of the polypeptide, such as, for example, by blocking the binding of substrates, enzymes, etc., and thereby alleviating the abnormal condition. In another approach, soluble forms of the polypeptides

10 still capable of binding the substrate, enzymes, etc. in competition with endogenous polypeptide may be administered. Typical examples of such competitors include fragments of the IGS5 polypeptide.

In still another approach, expression of the gene encoding endogenous IGS5

15 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered (see, for example, O'Connor, J. Neurochem. (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Alternatively, oligonucleotides which form triple helices ("triplexes")

20 with the gene can be supplied (see, for example, Lee et al., Nucleic Acids Res (1979) 6:3073; Cooney et al., Science (1988) 241:456; Dervan et al., Science (1991) 251:1360). These oligomers can be administered per se or the relevant oligomers can be expressed in vivo. Synthetic antisense or triplex oligonucleotides may comprise modified bases or modified backbones. Examples of the latter include methylphosphonate, phosphorothioate or peptide nucleic acid backbones. Such backbones are incorporated in the

25 antisense or triplex oligonucleotide in order to provide protection from degradation by nucleases and are well known in the art. Antisense and triplex molecules synthesized with these or other modified backbones also form part of the present invention.

30 In addition, expression of the IGS5 polypeptide may be prevented by using ribozymes specific to the IGS5 mRNA sequence. Ribozymes are catalytically active RNAs that can be natural or synthetic (see for example Usman, N, et al., Curr. Opin. Struct. Biol (1996) 6(4), 527-33.) Synthetic ribozymes can be designed to specifically cleave IGS5 mRNAs at selected positions thereby preventing translation of the IGS5 mRNAs into functional

polypeptide. Ribozymes may be synthesized with a natural ribose phosphate backbone and natural bases, as normally found in RNA molecules. Alternatively the ribozymes may be synthesized with non-natural backbones to provide protection from ribonuclease degradation, for example, 2'-O-methyl RNA, and may contain modified bases.

5

For treating abnormal conditions related to an under-expression of IGS5 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which stimulates a polypeptide of the present invention in combination with a pharmaceutically acceptable carrier, to
10 thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of IGS5 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a
15 retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells in vivo and expression of the polypeptide in vivo. For an overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches,
20 (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of a polypeptide of the present invention in combination with a suitable pharmaceutical carrier.

Formulation and Administration

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide, such as the soluble form of a polypeptide of the present invention, stimulating or inhibiting peptide or small molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, and the like.

The dosage range required depends on the choice of peptide or other compounds of the present invention, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide ex vivo, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Polynucleotide and polypeptide sequences form a valuable information resource with which it is possible to identify further sequences of similar homology. This is most easily facilitated by storing the sequence in a computer readable medium and then using the stored data to search a sequence database using well known searching tools, such as those in the GCC and Lasergene software packages. Accordingly, in a further aspect, the present invention provides for a computer readable medium having stored thereon a polynucleotide comprising the sequences of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5 and/or a polypeptide sequence encoded thereby.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The following examples are only intended to further illustrate the invention, in more detail, and therefore these examples are not deemed to restrict the scope of the invention in any way.

**EXAMPLE 1. THE CLONING OF cDNA ENCODING A NOVEL MEMBER OF THE
NEP/ECE METALLOPROTEASE FAMILY.**

**5 Example 1a. Homology PCR cloning of a cDNA fragment of a novel member of the
NEP/ECE metalloprotease family.**

In the DNA databank of expressed sequence tags (ESTs) 4 overlapping EST sequences
(accession nos. AA524283, AI088893, AI217369 and AI380811) were detected which
10 contained a small open reading frame encoding a stretch of protein that showed similarity
to the C-terminal part of members of the neutral endopeptidase 24.11 / endothelin
converting enzyme (NEP/ECE) metalloprotease protein family (Turner A.J. et al. Faseb J.
[1997] 11: 355-364). The NEP/ECE-like small open reading frame in these ESTs was
terminated by a stop codon (in the case of AA524283) and was preceded in all 4 ESTs by
15 a sequence that contained stop codons in all 3 reading frames. This preceding sequence
appeared totally unrelated to NEP/ECE metalloprotease family members. Although the
polarity of the small open reading frame was opposite to the 5'→ 3' orientation of the
mRNA from which these ESTs had been derived, it was decided to use these sequences
as the basis for a RT-PCR homology cloning approach. In parallel, additional EST
20 sequences, that showed the same structure as the 4 ESTs mentioned before, were
observed to appear in the public domain databanks, e.g. accession nos: AI825876,
AI888306, AI422224, AI422225, AI469281, AA975272, AA494534, AW006103,
AI827701, AI650385, AI827898, AI934499 and AA422157. The RT-PCR reactions were
carried out using a reverse primer (IP11689; SEQ ID NO:7) designed on the EST cluster
25 (within the area showing similarity to the NEP/ECE family) and a degenerated forward
primer (IP11685; SEQ ID NO:8), centered on a conserved peptide motif [VNA(F,Y)Y] of
the NEP/ECE family.

For the synthesis of cDNA 2 µg human lung total RNA (Clontech #64023-1), 1 µl
30 oligo(dT)₁₂₋₁₈ (500 µg/ml) and 9 µl H₂O were combined [final volume = 12 µl], heated to
70 °C for 10 minutes and then chilled on ice. 4 µl 5 x first strand buffer [250 mM Tris-HCl
pH 8.3, 375 mM KCl, 15 mM MgCl₂], 2 µl 0.1M DTT, 1 µl 10 mM dNTP mix and 1 µl (200
U) Superscript™ II (Life Technologies) reverse transcriptase were added. The mixture

was incubated at 42 °C for 50 minutes and the reaction was inactivated by heating at 70 °C for 15 minutes.

The PCR reaction was performed in a 50 µl volume containing 1 µl of the cDNA synthesis reaction, 5 µl of GeneAmp™ 10 x PCR buffer (500 mM KCl, 100 mM Tris pH 8.3, 15 mM MgCl₂, 0.01% (w/v) gelatin; Perkin Elmer) , 2 µl of 10 mM dNTP mix, 10 pmoles each of the forward and reverse primers and 5 units AmpliTaq™ polymerase (Perkin Elmer). After an initial denaturation at 95 °C for 5 min., PCR reactions were cycled 40x as follows: 1 min denaturation at 94 °C, 1 min annealing at 60 °C and 1 min extension at 72 °C. PCR reaction products were analyzed by agarose gel electrophoresis. The IP11685/IP11689 RT-PCR reaction produced an amplicon of ± 600 base pairs (bp). The fragment was purified from gel using the Qiaex-II™ purification kit (Qiagen) and ligated into the pGEM-T Easy plasmid according to the procedure recommended by the supplier (pGEM-T Easy system, Promega). The recombinant plasmids were then used to transform competent E.coli SURE™ 2 bacteria (Stratagene). Transformed cells were plated on LB agar plates containing ampicillin (100 µg/l), IPTG (0.5 mM) and X-gal (50 µg/ml). Plasmid DNA was purified from mini-cultures of individual colonies using the BioRobot™ 9600 nucleic acid purification system (Qiagen). DNA Sequencing reactions were carried out on the purified plasmid DNA with the ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready Reaction kit (PE-ABI), using insert-flanking or internal (IGS5 specific) primers. Plasmid inserts were completely sequenced on both strands. Cycle Sequencing reaction products were purified via EtOH/NaOAc precipitation and analyzed on an ABI 373 automated sequencer. The DNA sequence of the inserts of recombinant clones YCE14, YCE15 and YCE16 (derived from the IP11685/IP11689 amplicon) extended the open reading frame of the original EST cluster in the direction of the N-terminus and further confirmed that this open reading frame was derived from a novel member of the NEP/ECE metalloprotease protein family (see Fig. 1). This upstream sequence thus deviated completely from the upstream sequence present in the EST sequences. This novel sequence is referred to within the context of the present invention generally as "IGS5".

30

Example 1b. Cloning of cDNA containing the putative ectodomain of IGS5.

In order to obtain additional IGS5 cDNA sequence another round of RT-PCR reactions

were carried out on human lung RNA under the conditions described above using the IGS5 specific reverse primer IP12190 (SEQ ID NO:9) and a degenerated forward primer (IP12433; SEQ ID NO:10), centered on a conserved peptide motif [LXXLXWMD] of the NEP/ECE family. The IP12190/12433 RT-PCR reaction produced an amplicon of \pm 600
5 bp that was cloned into the pGEM-T Easy vector yielding clones YCE19, YCE22 and YCE23. All clones were fully sequenced and allowed to extend the IGS5 open reading frame further upstream (see Fig.1).

To obtain cDNA clones that would cover the 5' end of the IGS5 transcript, semi-nested 5'-
10 RACE PCR reactions were done on human heart Marathon-Ready™ cDNA using the adaptor primer 1 (AP1: SEQ ID NO:11) provided with the Marathon™ cDNA amplification kit (Clontech K1802-1) in combination with IGS5 specific primers IP12189 SEQ ID NO:12) and IP12585 (SEQ ID NO:13). PCR RACE reactions were performed according to the instructions of the Marathon-Ready™ cDNA user manual provided by Clontech. RACE
15 products were separated on agarose gel, visualized with ethidium bromide and blotted onto Hybond N⁺ membranes. Blots were prehybridized at 65 °C for 2 h in modified Church buffer (0.5 M phosphate, 7% SDS, 10 mM EDTA) and then hybridized overnight at 65 °C in the same buffer containing 2×10^6 cpm/ml of the ³²P-labelled insert of clone YCE23. The YCE23 insert was radiolabelled via random primed incorporation of [α -³²P]dCTP to a
20 specific activity of $> 10^9$ cpm/ μ g using the Prime-It II kit™ (Stratagene) according to the instructions provided by the supplier. Hybridized blots were washed at high stringency (2 x 30 min at room temperature in 2 x SSC/0.1% SDS followed by 2 washes of 40 min at 65 °C in 0.1 x SSC, 0.1% SDS) and autoradiographed overnight. Hybridizing fragments were purified from gel, cloned into the pGEM-T Easy vector (yielding clones YCE 59, YCE
25 64 and YCE 65) and sequenced as described above.

The DNA sequences of all isolated clones could be assembled into a single contig (IGS5CONS; see Fig.1) that extended the open reading frame of IGS5 further upstream but an ATG start of translation codon was not yet encountered. Primer IP11689 had been
30 designed on EST AI380811 and did not contain the last 4 nucleotides before the stop codon present in the aligned EST sequences. In order to generate an open reading frame that terminated at the stop codon the last (consensus) 22 nucleotides of the aligned EST sequences were included in the overall assembly of IGS5CONS.

Homology searches showed that the (partial) encoded protein was most similar to neutral endopeptidase (NEP; see example 2). However, the initial 20 amino acids of the IGS5CONS open reading frame did not show any similarity to NEP. This could possibly be due to the fact that they were derived from an intron. Indeed exon 4 of human NEP starts at a position that corresponds approximately to the position downstream of these 20 amino acids (D'Adamio L. et al. Proc. Natl. Acad. Sci. USA [1989] 86: 7103-7107). Hydropathy analysis (Kyte J. et al. [1982] J. Mol. Biol. 157: 105-132; Klein P. et al. [1985] Biochim. Biophys. Acta 815:468-476) did not indicate the presence of a transmembrane domain within the predicted IGS5CONS amino acid sequence, although such a transmembrane domain would be expected to occur (or at least overlap with) within the initial 20 amino acids. For these reasons it was preferred to exclude the initial sequence part of the IGS5 contig (Fig.1). The resulting DNA sequence (IGS5DNA; SEQ ID NO:1) is 2076 nucleotides long (including the stop codon) and encodes a protein of 691 residues (IGS5PROT, SEQ ID NO:2). Alignment of IGS5PROT with the human NEP protein sequence showed that the IGS5PROT sequence corresponds to the complete ectodomain sequence of NEP. IGS5PROT can thus be expected to carry the complete enzymatic activity of the putative IGS5 enzyme, as was demonstrated for the ectodomain of NEP (Fossiez F. et al. Biochem. J. [1992] 284, 53-59).

20

Table 7: Overview of oligo primers used in Example 1

SEQ ID NO:7	IP11689:	5' -ACACGGCATCGCTCCTTG-3'
SEQ ID NO:8	IP11685:	5' -CCCCCTGGACGGTGAA(C or T)GC(A,C,G or T)T(A or T)(C or T)TA-3'
SEQ ID NO:9	IP12190:	5' -AATCCGTTACGTTCTGTTTCGTCTGCC-3'
SEQ ID NO:10	IP12433:	5' -CCTGGAGGAGCTG(A,C or G)(A,C or T)(A,C, G or T)TGGATG(A or G)A-3'
SEQ ID NO:11	AP1:	5' -CCATCCTAATACGACTCACTATAGGGC-3'
SEQ ID NO:12	IP12189:	5' -GTCCTTGCCACCCTCTGCCATCC-3'
SEQ ID NO:13	IP12585:	5' -ACCACCCCCGCCCGATGATCCAGAG-3'

EXAMPLE 2. ALIGNMENT OF IGS5 WITH PROTEIN SEQUENCES OF MEMBERS OF THE NEP/ECE METALLOPROTEASE FAMILY.

For the IGS5 Sequence cloned in example 1a, homology searches of up to date protein
5 databanks and translated DNA databanks were executed using the BLAST algorithm
(Altschul S.F. et al. [1997], Nucleic Acids Res. 25:3389-3402). These searches showed
that the IGS5 protein was most similar (54-55% identities over \pm 700 aligned residues) to
mouse, rat and human neutral endopeptidase (SW:NEP_MOUSE, accession n° Q61391;
SW:NEP_RAT, accession n° P07861 and SW:NEP_HUMAN accession n° P08473).

10

Thus, this alignment of the almost complete IGS5 protein sequence with the other
members of the NEP/ECE family shows the relation of IGS5 to metalloproteases in
general, and in particular to the NEP and/or ECE metalloprotease families. From this
structural alignment it is concluded that the IGS5 has the functionality of metallo-
15 proteases, which in turn are of interest in the context of several dysfunctions, disorders or
diseases in animals and humans.

EXAMPLE 3. THE CLONING OF cDNA ENCODING A NOVEL MEMBER OF THE 20 NEP/ECE METALLOPROTEASE FAMILY.

Example 3a. Homology PCR cloning of a cDNA fragment of a novel member of the NEP/ECE metalloprotease family.

25 In the DNA databank of expressed sequence tags (ESTs) 4 overlapping EST sequences
(accession nos. AA524283, AI088893, AI217369 and AI380811) were detected which
contained a small open reading frame encoding a stretch of protein that showed similarity
to the C-terminal part of members of the neutral endopeptidase 24.11 / endothelin
converting enzyme (NEP/ECE) metalloprotease protein family (Turner A.J. et al., Faseb J.
30 [1997] 11: 355-364). The NEP/ECE-like small open reading frame in these ESTs was
terminated by a stop codon (in the case of AA524283) and was preceded in all 4 ESTs by
a sequence that contained stop codons in all 3 reading frames. This preceding sequence
appeared totally unrelated to NEP/ECE metalloprotease family members. Although the
polarity of the small open reading frame was opposite to the 5'->3' orientation of the

mRNA from which these ESTs had been derived, it was decided to use these sequences as the basis for a RT-PCR homology cloning approach. In parallel, additional EST sequences, that showed the same structure as the 4 ESTs mentioned before were observed to appear in the public domain databanks, e.g. accession nos: AI825876, AI888306, AI422224, AI422225, AI469281, AA975272, AA494534, AW006103, AI827701, AI650385, AI827898, AI934499 and AA422157. The RT-PCR reactions were carried out using a reverse primer (IP11689; SEQ ID NO:7) designed on the EST cluster (within the area showing similarity to the NEP/ECE family) and a degenerated forward primer (IP11685; SEQ ID NO:8), centered on a conserved peptide motif [VNA(F,Y)Y] of the NEP/ECE family.

For the synthesis of cDNA 2 µg human lung total RNA (Clontech #64023-1), 1 µl oligo(dT)₁₂₋₁₈ (500 µg/ml) and 9 µl H₂O were combined (final volume = 12 µl), heated to 70 °C for 10 minutes and then chilled on ice. 4 µl 5 x first strand buffer (250 mM Tris-HCl pH 8.3, 375 mM KCl, 15 mM MgCl₂), 2 µl 0.1 M DTT, 1 µl 10 mM dNTP mix and 1 µl (200 U) Superscript™ II (Life Technologies) reverse transcriptase were added. The mixture was incubated at 42 °C for 50 minutes and the reaction was inactivated by heating at 70 °C for 15 minutes.

The PCR reaction was performed in a 50 µl volume containing 1 µl of the cDNA synthesis reaction, 5 µl of GeneAmp™ 10 x PCR buffer (500 mM KCl, 100 mM Tris pH 8.3, 15 mM MgCl₂ 0.01% (w/v) gelatin; PE Biosystems), 2 µl of 10 mM dNTP mix, 10 pmoles each of the forward and reverse primers and 5 units AmpliTaq™ polymerase (PE Biosystems). After an initial denaturation at 95 °C for 5 min., PCR reaction tubes were cycled 40x as follows: 1 min denaturation at 94 °C, 1 min annealing at 60 °C and 1 min extension at 72 °C. PCR reaction products were analyzed by agarose gel electrophoresis. The IP11685/IP11689 RT-PCR reaction produced an amplicon of ± 600 base pairs (bp). The fragment was purified from gel using the Qiaex-II™ purification kit (Qiagen) and ligated into the pGEM™-T Easy plasmid according to the procedure recommended by the supplier (pGEM™-T Easy system, Promega). The recombinant plasmids were then used to transform competent E.coli SURE™ 2 bacteria (Stratagene). Transformed cells were plated on LB agar plates containing ampicillin (100 µg/ml), IPTG (0.5 mM) and X-gal (50 µg/ml). Plasmid DNA was purified from mini-cultures of individual colonies using the BioRobot™ 9600 nucleic acid purification system (Qiagen). DNA sequencing reactions

were carried out on the purified plasmid DNA with the ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready Reaction kit (PE Biosystems), using insert-flanking or internal primers. Plasmid inserts were completely sequenced on both strands. Cycle Sequencing reaction products were purified via EtOH/NaOAc precipitation and analyzed on an ABI 377 automated sequencer. The DNA sequence of the inserts of recombinant clones YCE14, YCE15 and YCE16 (derived from the IP11685/IP11689 amplicon) extended the open reading frame of the original EST cluster in the direction of the N-terminus and further supported the hypothesis that this open reading frame was derived from a novel member of the NEP/ECE metalloprotease protein family (Fig.2). This upstream sequence thus deviated completely from the upstream sequence present in the EST sequences. This novel sequence is referred to within the context of the present invention generally as "IGS5".

Example 3b. Cloning of cDNA fragments containing the full length coding sequence of IGS5.

In order to obtain additional IGS5 cDNA sequence another round of RT-PCR reactions were carried out on human lung RNA under the conditions described above using the IGS5 specific reverse primer IP12190 (SEQ ID NO:9) and a degenerated forward primer (IP12433; SEQ ID NO:10), centered on a conserved peptide motif [LXXLXWMD] of the NEP/ECE protein family. The IP12190/12433 RT-PCR reaction produced an amplicon of ± 600 bp that was cloned into the pGEM™-T Easy vector yielding clones YCE19, YCE22 and YCE23. All clones were fully sequenced and allowed to extend the IGS5 open reading frame further upstream (see Fig.2).

To obtain cDNA clones that would cover the 5' end of the IGS5 transcript, semi-nested 5'-RACE PCR reactions were done on human heart Marathon-Ready™ cDNA using the adaptor primer 1 (AP1: SEQ ID NO:11) provided with the Marathon™ cDNA amplification kit (Clontech K1802-1) in combination with IGS5 specific primers IP12189 (SEQ ID NO:12) and IP12585 (SEQ ID NO:13). PCR RACE reactions were performed according to the instructions of the Marathon-Ready™ cDNA user manual provided by Clontech. RACE products were separated on agarose gel, visualized with ethidium bromide and blotted onto Hybond™-N+ membranes (Amersham). Blots were prehybridized at 65 °C for

2 h in modified Church buffer (0.5 M phosphate, 7% SDS, 10 mM EDTA) and then hybridized overnight at 65 °C in the same buffer containing 2×10^6 cpm / ml of the ^{32}P -labelled insert of clone YCE23. The YCE23 insert was radiolabelled via random primed incorporation of $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ to a specific activity of $> 10^9$ cpm/ μg using the Prime-It II kitTM (Stratagene) according to the instructions provided by the supplier. Hybridized blots were washed at high stringency (2 x 30 min at room temperature in 2 x SSC/0.1% SDS followed by 2 washes of 40 min at 65 °C in 0.1 x SSC, 0.1% SDS) and autoradiographed overnight. Hybridizing fragments were purified from gel, cloned into the pGEMTM-T Easy vector (yielding clones YCE 59, YCE 64 and YCE 65) and sequenced as described above.

The DNA sequences of all isolated clones could be assembled into a single contig that extended the open reading frame of IGS5 further upstream although no start of translation codon was yet encountered. Primer IP11689 had been designed on EST AI380811 and did not incorporate the last 4 nucleotides before the stop codon present in the aligned EST sequences. In order to generate an open reading frame that terminated at this stop codon the last (consensus) 22 nucleotides of the aligned EST sequences were included in the contig.

Several attempts to clone the still missing amino-terminal part of the IGS5 coding sequence via 5' RACE PCR extension or via screening of cDNA libraries failed. Therefore it was tried to obtain genomic sequence information in the area around and upstream of the 5' end of the preliminary IGS5 contig. Approximately 550,000 plaques of a human genomic DNA library, constructed in the lambda EMBL3 phage vector (Clontech HL1067j) were lifted onto HybondTM-N⁺ membranes. Membrane lifts were prehybridized at 65 °C for 2 h in modified Church buffer and then hybridized overnight at 65 °C in the same buffer containing 2×10^6 cpm/ml of a ^{32}P -labeled ± 150 bp *EcoRI*/*EcoRII* fragment, located at the 5' end of clone YCE59. The cDNA probe was radiolabelled via random primed incorporation of $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ to a specific activity of $> 10^9$ cpm/ μg using the Prime-It II kitTM (Stratagene) according to the instructions provided by the supplier. Hybridized membranes were washed at high stringency (2 x 30 min at room temperature in 2 x SSC/0.1% SDS followed by 1 wash of 40 min at 65°C in 0.1 x SSC/0.1% SDS) and autoradiographed. Hybridizing plaques were subjected to a second round of screening and pure single plaques were obtained. Recombinant phage DNA was purified from

infected liquid cultures using the Qiagen™ Lambda Midi Kit (Qiagen) and sequenced as described above using flanking EMBL3 vector primers and IGS5 internal primers. From the insert of clone IGS5/S1 approximately 5,000 nucleotides upstream of the 5' end of the preliminary IGS5 contig were sequenced. Homology searches of translated DNA databanks showed that this 5,000 bp fragment contained a stretch of 78 bp which encoded a peptide that was most similar (15 identical residues over 25 aligned) to an alternatively spliced 69 bp fragment in the mouse SEP sequence (GenBank accession no AF157105), which is a recently described novel member of the NEP/ECE family (Ikeda et al. [1999] JBC 274: 32469-32477). This 78 bp human fragment was preceded by and followed by putative consensus splice acceptor and donor sites respectively but did not contain an "ATG" start of translation codon.

In order to obtain cDNA clones containing the amino-terminal part of the IGS5 coding sequence, semi-nested 5' RACE PCR reactions were carried out on human testis Marathon-Ready™ cDNA (Clontech 7414-1) using the adapter primer 1 (AP1: SEQ ID NO:11) provided with the Marathon™ cDNA amplification kit (Clontech K1802-1) in combination with IGS5 specific anti-sense primers IP14,241 (SEQ ID NO:14) and IP14242 (SEQ ID NO:15) which were designed within the 78 bp genomic fragment described above. PCR RACE reactions were performed according to the instructions of the Marathon Ready™ cDNA user manual provided by Clontech (reaction volume = 25 µl). RACE products were separated on agarose gel, visualised with ethidium bromide and analyzed via Southern blot.

To generate a specific hybridization probe for the blotted RACE products, a semi-homology PCR reaction was carried out on the above obtained nested RACE products using the reverse oligonucleotide primer IP14241 (SEQ ID NO:14) and a degenerated forward primer (IP13798; SEQ ID NO:16) which was centered on a peptide motif [GLMVLLLL] within the transmembrane domain of the mouse SEP protein. The PCR reaction was performed in a 25 µl volume containing 1 µl of the semi-nested 5' RACE PCR reaction product, 2.5 µl of GeneAmp™ 10 x PCR buffer (500 mM KCl, 100 mM Tris pH 8.3, 15 mM MgCl₂, 0.01% (w/v) gelatin; PE Biosystems), 1 µl of 10 mM dNTP mix, 10 pmoles each of the forward and reverse primers and 2.5 units AmpliTaq-Gold™ polymerase (PE Biosystems). After an initial denaturation at 95 °C for 10 min, PCR reaction tubes were cycled 35x as follows: 1 min denaturation at 95 °C, 30 seconds

annealing at 50 °C and 30 seconds extension at 72 °C. PCR reaction products were analyzed via agarose gel electrophoresis. The semi-homology PCR reaction produced an amplicon of ± 110 base pairs. The fragment was purified from gel using the Qiaex-II™ purification kit (Qiagen) and ligated into the pGEM™-T plasmid according to the procedure recommended by the supplier (pGEM™-T system, Promega). The recombinant plasmids were then used to transform competent *E.coli* SURE™ 2 bacteria (Stratagene). Transformed cells were plated on LB agar plates containing ampicillin (100µg/l), IPTG (0.5 mM) and X-gal (50 µg/ml). Plasmid DNA was purified from mini-cultures of individual colonies using the BioRobot™ 9600 nucleic acid purification system (Qiagen) and sequenced as described above. The DNA sequence of the inserts of recombinant clones YCE207, YCE212, YCE216, YCE217, YCE218 and YCE219 could be assembled with the 78 bp genomic fragment described above into a single contig (see Fig. 2).

Southern blots of the semi-nested 5' RACE PCR reaction products were prehybridized at 65 °C for 1 h in modified Church buffer and then hybridized overnight at 65 °C in the same buffer containing 2×10^8 cpm/ml of the ³²P-labelled insert of clone YCE207. Hybridized blots were washed at high stringency and autoradiographed. Hybridizing fragments were purified from gel, cloned into the pGEM™-T vector (yielding clones YCE223, YCE224 and YCE226) and sequenced as described above. The DNA sequences of these clones could be assembled with the 78 bp genomic fragment and with clones YCE207, YCE212, YCE216, YCE217, YCE218 and YCE219 into a single contig (Fig. 2). The resulting contig contained an open reading frame which started at an "ATG" initiation codon and encoded a protein which showed high similarity with the N-terminal sequence of the mouse SEP protein.

To obtain cDNA clones covering the amino-terminal part of the IGS5 coding sequence and overlapping with clone YCE59, PCR reactions were set up on human testis Marathon-Ready™ cDNA (Clontech 7414-1) using a specific forward primer (IP14535; SEQ ID NO:17) based on the 5' UTR sequence of IGS5 and a specific reverse primer (IP14537; SEQ ID NO:18) located within YCE59. The PCR reaction was performed in a 25 µl volume containing 2.5 µl of human testis Marathon-Ready™ cDNA, 2.5 µl of GeneAmp™ 10 x PCR buffer (500 mM KCl, 100 mM Tris pH 8.3, 15 mM MgCl₂, 0.01% (w/v) gelatin; PE Biosystems), 1 µl of 10 mM dNTP mix, 10 pmoles each of the forward and reverse primers and 2.5 units AmpliTaq-Gold™ polymerase (PE Biosystems). After

an initial denaturation at 95 °C for 10 min., PCR reaction tubes were cycled 41x as follows: 1 min denaturation at 95 °C, 1 min annealing at 53 °C and 1 min extension at 72 °C. PCR reaction products were analysed by agarose gel electrophoresis. The PCR reaction produced two amplicons of \pm 300 and 380 base pairs respectively. The 300 bp and 380 bp fragments were purified from gel, cloned into the pGEM™-T vector and sequenced as described above. This yielded clones YCE231, YCE233 and YCE235 (300 bp fragment) and YCE229 (380 bp fragment).

Assembly of the DNA sequences of all isolated clones showed the presence of two types of cDNA sequences, that differed by the presence or absence of the 78 bp segment, initially identified within genomic clone IGS5/S1. These two sequences likely originate from alternatively spliced RNA molecules. The longest transcript contains an open reading frame of 2337 nucleotides (encoding a protein of 779 residues) whereas the shorter transcript contains an open reading frame of 2259 nucleotides (encoding a protein of 753 residues). We refer to the coding sequence and protein sequence of the long form as IGS5DNA1 (shown in SEQ ID NO:3, 2340 bp including the stop codon tag) and IGS5PROT1 (SEQ ID NO:4) respectively, whereas the coding sequence and protein sequence of the shorter form are referred to as IGS5DNA2 (shown in SEQ ID NO:5, 2262 bp including the stop codon tag) and IGS5PROT2 (SEQ ID NO:6) respectively. Downstream of the postulated methionine initiation codon within IGS5DNA1 and IGS5DNA2 an additional in-frame methionine codon is present at codon position 10. Although we have opted for the first methionine codon as being the initiation codon some (or even exclusive) initiation of translation at codon position 10 cannot be excluded, since both methionines appear to be within an equally favorable "Kozak" initiation of translation context (Kozak M., Gene [1999]: 234: 187-208). Hydropathy analysis (Kyte J. et al., J. Mol. Biol. [1982] 157: 105-132; Klein P. et al., Biochim. Biophys. Acta [1985] 815: 468-476) of the IGS5PROT1 and IGS5PROT2 sequences showed the presence of a single transmembrane domain between residues 22 to 50. This indicates that IGS5PROT1 and IGS5PROT2 are type II integral membrane proteins and thus have a membrane topology similar to other members of the NEP/ECE protein family.

Table 8: Overview of the oligonucleotide primers that were used in Example 3.

SEQ ID NO:7	IP11689:	5' -ACACGGCATCGCTCCTTG-3'
SEQ ID NO:8	IP11685:	5' -CCCCCTGGACGGTGAA (C or T) GC (A,C,G or T) T (A or T) (C or T) TA-3'
SEQ ID NO:9	IP12190:	5' -AATCCGTTACAGTTCTGTTCGTCTGCC-3'
SEQ ID NO:10	IP12433:	5' -CCTGGAGGAGCTG (A,C or G) (A,C or T) (A,C,G or T) TGGATG (A or G) A-3'
SEQ ID NO:11	AP1:	5' -CCATCCTAATACGACTCACTATAGGGC-3'
SEQ ID NO:12	IP12189:	5' -GTCCTTGCCACCCTCTGCCATCC-3'
SEQ ID NO:13	IP12585:	5' -ACCACCCCCGCCCCGATGATCCAGAG-3'
SEQ ID NO:14	IP14241:	5' -ACAGCCGGCTAGCAAGGCGTGGCAGCTG-3'
SEQ ID NO:15	IP14242:	5' -ACGACAGCCGGCTAGCAAGGCGTGGCAG-3'
SEQ ID NO:16	IP13798:	5' -GG (A,C,G or T) CT (C or G) ATGGT (A,C,G or T) CT (C or G) CT (C or G) CT (C or G) CT (C or G) -3'
SEQ ID NO:17	IP14535:	5' -CTCCTGAGTGAGCAAAGGTTCC-3'
SEQ ID NO:18	IP14537:	5' -GCAAAGTGGTAGAAGTCGTCACAC-3'

5 EXAMPLE 4. ALIGNMENT OF IGS5 WITH PROTEIN SEQUENCES OF MEMBERS OF THE NEP/ECE METALLOPROTEASE FAMILY.

For the IGS5 sequence cloned in example 3, homology searches of up to date protein databanks and translated DNA databanks were executed using the BLAST algorithm (Altschul S.F. et al, Nucleic Acids Res. [1997] 25:3389-3402). These searches showed that IGS5PROT1 was most similar (76% identities over 778 aligned residues) to mouse SEP (GenBank accession n° AF157105) and also showed 54-55 % identities over 696 aligned residues to mouse, rat and human neutral endopeptidases (SW:NEP_MOUSE, accession n° Q61391; SW:NEP_RAT, accession n° P07861; SW:NEP_HUMAN, accession n° P08473). Homology searches of IGS5PROT2 showed that this sequence was most similar (78% identities over 752 aligned residues) to mouse SEP (GenBank accession no AF157106). In analogy with the mouse SEP and SEP proteins it is to be

expected that IGS5PROT1 and IGS5PROT2 represent the membrane-bound and soluble forms of the IGS5 protein respectively. This is corroborated by the presence of dibasic residues (KRK) encoded at the 3' end of the the alternatively spliced 78bp exon.

5 Thus, this alignment of the complete IGS5 protein sequence with the other members of the NEP/ECE family shows the relation of IGS5 to NEP/ECE metalloproteases in general, and in particular to the SEP and NEP family members. From this structural alignment it is concluded that the IGS5 protein has the functionality of metalloproteases, which in turn are of interest in the context of several dysfunctions, disorders or diseases in animals and
10 humans.

EXAMPLE 5. RNA EXPRESSION ANALYSIS OF IGS5.

15 **IGS5 expression analysis on Human RNA Master Blot™.** A solution of Express-Hyb™ (Clontech #8015-1) and sheared salmon testis DNA was prepared as follows: 15 ml of Express-Hyb was preheated at 50-60°C. 1.5 mg of sheared salmon testis DNA was heated at 95°C for 5 minutes and then quickly chilled on ice. The heat-denatured sheared salmon testis DNA was mixed with the preheated Express-Hyb™. The human RNA
20 Master Blot™ (Clontech #7770-1) was prehybridised in 10 ml of the solution prepared above for 30 minutes with contiguous agitation at 65°C. The ³²P labelled YCE15 probe (labelled with Prime-it II™ kit, Stratagene) was heat-denatured and added to the remaining 5 ml of the Express-Hyb™ solution. Hybridisation was done overnight at 65°C. Washings were done in 2 x SSC/1% SDS for 100 minutes (5 x 20 min.) at 65°C. Two
25 additional 20 minutes washes were performed in 200 ml 0.1 x SSC/0.5% SDS at 55°C. Finally the Master Blot was autoradiographed using X-ray film. Hybridization of the IGS5 probe on the Master Blot™ showed expression in a wide range of tissues, and in particular expression in testis, small intestine, prostate and stomach (Fig. 3).

30

IGS5 expression analysis on Human Brain Multiple Tissue Northern Blots II and IV (#7755-1 and #7769-1 respectively). An Express-Hyb™ solution (Clontech #8015-1) was preheated at 68°C. The blot was prehybridised at 68°C for 1 hour. 100 µg sheared salmon testis DNA was added to the ³²P labelled YCE15 probe (labelled with Prime-it II™

kit, Stratagene) and heat-denatured at 95°C for 10 minutes. The probe was added to the remaining 5 ml of the Express-Hyb™ solution and hybridisation was done for 2 hours at 68°C. Washings were done in 2 x SSC/0.05% SDS for 40 minutes (2 x 20 min.) at RT. Two additional 20 minutes washes were performed in 200 ml 0.1 x SSC/0.1% SDS at 55°C. The blot was autoradiographed using X-ray film.

This Northern blot analysis showed a major hybridizing band of ± 3 kb and a minor band of ± 5.5 -6 kb in all tissues investigated.

10 **EXAMPLE 6. EXPRESSION AND PURIFICATION OF THE HIS-TAGGED ECTODOMAIN OF HUMAN IGS5.**

The aim of the experiment was to produce soluble IGS5 protein using the baculoviral expression system. A recombinant baculovirus was constructed that expressed the His₆-tagged IGS5 ectodomain upon infection of the Sf9 cell-line. Soluble IGS5 protein was then purified from the culture supernatant in a two step procedure involving lentil-lectin and Zn-IMAC chromatography.

We fused the signal peptide of the pro-opiomelanocortin precursor (POMC) to the His-tagged extracellular part of the IGS5 coding sequence. As the enzymatically active site (metalloprotease) of the protein is located at the C-terminal end, we preferred to add the His-purification tag at the N-terminus of the protein. Furthermore a Gly- Ser linker was inserted between the POMC signal peptide and the IGS5 ectodomain. The expressed IGS5 protein started at residue 60 of IGS5PROT2 (SEVC...) and thus comprised almost the complete IGS5 ectodomain. The cloning strategy involved a combination of synthetic oligonucleotide assembly, overlap PCR and 3-points-ligation. This resulted in the expression of a protein consisting of the POMC signal (cleaved upon secretion), a Gly-Ser linker, a His₆ peptide and the IGS5 extracellular domain.

Example 6a. Construction of the pAcSG2SOLhuIGS5His6 baculo transfer vector.

For the construction of the pAcSG2SOLhuIGS5 baculo transfer vector the following DNA
5 fragments were generated:

1. The pAcSG2 vector (BD PharMingen) was *Stu*I/*Not*I digested. The 5527bp fragment
was extracted from agarose gel using the QiaExII extraction kit (Westburg) and
dissolved in 30µl 10mM Tris-HCl pH8.5.
10
2. pGEMT clone YCE174 was assembled from clones YCE15, YCE22, YCE64 and
YCE65 via a combination of PCR and restriction digestion/ligation. Primer IP13541,
which in contrast to IP11689 did contain the last 4 nucleotides of the IGS5 coding
sequence and the stop codon, was used in this procedure (Table 9). YCE174
15 therefore contained almost the complete coding region of the huIGS5 extracellular
domain down to (and including) the stop codon (Fig.2). YCE174 was *Xho*I/*Not*I
digested resulting in a 3025bp, a 1723bp and a 448bp fragment as shown by agarose
gel electrophoresis. The 1723bp fragment, containing the coding region for the
huIGS5 ectodomain, was extracted from gel (QiaexII, Qiagen) and dissolved in 20µl
20 10mM Tris-HCl pH8.5.
3. A synthetic nucleic acid fragment (180bp) containing a *Stu*I recognition site at the 5'
end, followed by the POMC signal sequence, a Gly-Ser linker, a His6 tag and 65 bp of
the 5' end of the IGS5 ectodomain coding sequence was assembled by combining the
25 oligonucleotides IP14165, IP14114, IP14115, IP14116, IP14117, IP14118, IP14119,
and IP14120, followed by overlap PCR with primers IP14166 and IP14110 (Table 9;
see also Fig.4). The *Stu*I site present in the natural POMC signal peptide coding
sequence was removed by introducing a silent mutation (IP14115, nucleotide 30 G ->
A) at bp position 57.
30

Table 9: Overview of the oligonucleotide primers that were used in Example 6.

SEQ ID NO:19	IP14165:	5' -GACAAGGCCTATTATGCCGAGATCGTGCTGCAGCCGCTCG - 3'
--------------	----------	---

SEQ ID NO:20	IP14114:	5' -AAGGCCAGCAACAGGGCCCCCGAGCGGCTGCAGCACGATC-3'
SEQ ID NO:21	IP14115:	5' -GGGGCCCTGTTGCTGGCCTTGCTGCTTCAAGCCTCCATGG-3'
SEQ ID NO:22	IP14116:	5' -GTGAGAACCGCCACGCACTTCCATGGAGGCTGAAGCAGC-3'
SEQ ID NO:23	IP14117:	5' -AAGTGCGTGGCGGTTCTCACCATCACCACCATCACAGCGA-3'
SEQ ID NO:24	IP14118:	5' -AGCCAGGGGTGGTGCAGACCTCGCTGTGATGGTGGTGATG-3'
SEQ ID NO:25	IP14119:	5' -GGTCTGCACCACCCCTGGCTGCGTGATAGCAGCTGCCAGG-3'
SEQ ID NO:26	IP14120:	5' -GGGTCCATGTTCTGGAGGATCCTGGCAGCTGCTATCACGC-3'
SEQ ID NO:27	IP14166:	5' -GACAAGGCCTATTATG-3'
SEQ ID NO:28	IP14110:	5' -GGGTCCATGTTCTG-3'
SEQ ID NO:29	IP14111:	5' -AGCGAGGTCTGCAC-3'
SEQ ID NO:30	IP14112:	5' -GTAGATGATGTGCCG-3'
SEQ ID NO: 31	IP13541:	5' -GCACTAGTCTTGGCTACCACACGCGGCATCGCTCCTTG-3'

A second PCR fragment (495bp) was amplified from the clone YCE174 template using primers IP14111 and IP14112. The first and second PCR product share a 65bp long overlapping region. By using a mixture of both PCR products as a template, an overlap PCR was performed with primers IP14166 and IP14112, generating a final 610bp PCR fragment. This fragment was purified on a QiaQuick Spin Column (Qiagen), *Stu*I/*Xho*I digested and the resulting 496 bp fragment was extracted from agarose gel (QiaexII).

The three DNA fragments (5527, 1723 and 496 bp) that were generated as described above, were combined in a ligation reaction. The ligation mixture was incubated overnight at 16°C and used to transform competent DH5alphaF' cells. The transformed bacteria were plated on LB agar/ampicillin plates (100µg/ml ampicillin). Plates were incubated overnight at 37°C. 30 random colonies were cultured in 5ml LB medium supplemented with 100µg/ml ampicillin. Plasmid DNA was prepared using the Biorobot™ 9600 Nucleic Acid Purification System (Qiagen) and screened via *Bam*HI digestion. 7 clones that displayed the correct restriction pattern were further analyzed by *Xho*I, *Stu*I, *Alw*NI, *Hind*III and *Hinc*II digestion and sequence analysis of the insert. One clone with the correct

restriction pattern and expected insert sequence, was finally selected and deposited in the culture collection (strainlist) as ICCG4502. (This clone contains a silent mutation at bp position 878 (G -> A) of the transfer vector.)

5 A sterile Qiagen Midi DNA prep (Westburg) was made from the deposited clone, which yielded 110 µg DNA. Restriction analysis by XhoI, AlwNI, StuI, HindIII and HincII digestion revealed the correct restriction pattern as shown by agarose gel electrophoresis. Sequence analysis confirmed the expected sequence. The map of the pAcSG2SOLhuIGS5His6 baculo transfer vector is shown in Fig.5.

10

Example 6b. Generation of a recombinant baculovirus for expression of soluble huIGS5His6.

15 A recombinant baculovirus, expressing the extracellular domain (minus a few AA) of the N-terminal His tagged human IGS5 was generated by cotransfection (Ca-phosphate transfection method) of the pAcSG2SOLhuIGS5His6 transfer vector DNA (ICCG 4502) in the host insect cell (*Spodoptera frugiperda* Sf9 cells) with the linearized genomic DNA of a modified version of the wild type baculoviral genome, (BaculoGold; Pharmingen catn° 21100 D) in the host insect cells (*Spodoptera frugiperda* Sf9 cells). The BaculoGold DNA
20 contains a lethal deletion and does not code for viable virus. Co-transfection of the BaculoGold DNA with a complementing Baculovirus transfer vector rescues the lethal deletion by homologous recombination. Using this approach 3 individual candidate recombinants were plaque-purified. All candidate recombinants were amplified.

25

Example 6c. Eukaryotic expression.

Kinetic expression analysis. Sf9 cells (IGCL 83.0), exponentially growing in suspension in spinner flasks at 27°C in TC100 medium (JRH Biosciences Catn° 56941),
30 supplemented with 10% inactivated Foetal Calf Serum (Gibco BRL Catn° 10 084 168), were collected by low speed centrifugation and seeded at $5 \cdot 10^5$ cells/Fk (25cm²) in serum-free TC100 medium. Candidate recombinant viral clones were added at a multiplicity of infection (MOI) of 3 pfu/cell and cell/virus cultures were subsequently incubated at 27°C. Conditioned medium (CM) was harvested at 24, 48 and 72 h post infection by 2

consecutive low speed centrifugations. Samples were analysed by SDS PAGE gel electrophoresis and Western blotting.

Western blot revealed a clear band at approximately 81 kDa in the CM of all candidate clones, corresponding to the theoretical Mr of the mature protein (81.2 kDa) (not shown).

- 5 Expression levels of all 3 clones peaked at 48-72 h post-infection. Clone 2 was selected for further amplification and was deposited as IGBV73. Optimal harvest time was set at 72h post infection.

Deglycosylation study. The soluble human IGS5 protein sequence contains 8 potential
10 N-glycosylation sites (Fig. 6). Since the purification protocol involves binding of the sugar residues on a lentil-lectin column, samples of CM of all candidate recombinant viral clones, harvested at 72h post infection, were used for a deglycosylation study with N-glycosidase F, to check whether the recombinant soluble His6IGS5 protein is indeed expressed as a glycosylated protein.

- 15 Samples were supplemented with SDS to a final concentration of 1% and incubated at 95°C for 5'. After addition of 1 volume of the 2x incubation buffer (250mM phosphate buffer, 50 mM EDTA, 5% N-octylglycoside, 1% 2-mercaptoethanol) and an additional 5' incubation time at 95°C, the sample was cooled to 37°C. 1U of N-glycosidase F (Boehringer mannheim, catn° 1 365 177) was added and after overnight incubation at
20 37°C, the sample was reduced with 100mM DTT (final concentration).

Western blot analysis of N-glycosidase F treated CM samples and non-treated controls show a shift in Mr when samples are deglycosylated (Fig. 7), demonstrating that the soluble human His-tagged IGS5 is expressed as a glycosylated protein.

25

Example 6d. Purification.

- Preparative production and sample pretreatment.** Sf9 cells (IGCL 83-2) exponentially growing in suspension in spinner flasks at 27 °C in TC100 medium (JRH Biosciences, cat
30 n° 56941) supplemented with 10% inactivated Foetal Calf Serum (Gibco BRL, cat n° 10 084 168) were collected by low speed centrifugation and resuspended at a density of $2 \cdot 10^6$ cells/ml in TC100 medium, supplemented with 0.013 TIU aprotinin/ml (Pentex). Recombinant virus IGBV73 was added to the cells at a multiplicity of infection (MOI) of 2.25 pfu/cell. The cell/virus suspension was subsequently incubated at 27°C in glass roller

bottles (3 x 500ml/1260cm²) for 72h. The CM (1.5 l) was then cleared from cells and cell debris by two consecutive low speed centrifugations. 1 tablet of EDTA free complete (EFC; Roche biochemicals, cat n° 1873580) was added to 300 ml cleared Baculo CM. HEPES, glycerol and Tween 20 were added to a final concentration of resp. 20 mM, 5%
5 (v/v) and 0.005 % (w/v). The pH of the CM was adjusted to 7.4 and the sample was filtrated (Durapore Membrane Filters 0.2 µ GV). All purification steps were performed at 4°C.

Lentil Lectin Chromatography. The baculo sample was loaded overnight at 0.3 ml/min on a 5 ml Lentil Lectin Sepharose resin in a C10/10 column (Pharmacia), which had been
10 equilibrated in buffer A (20 mM Hepes, pH7.4, 150 mM NaCl, 5% glycerol, 0.005% Tween 20) supplemented with 1 tablet EFC/ 500 mL. The column was washed with equilibration buffer until the absorbance at 280 nm reached baseline level and the bound proteins were eluted at 1ml/min by applying buffer A containing 0.5 M alfa-methylpyrannoside. The column was regenerated by applying 100 mM acetate, 500 mM
15 NaCl, pH 5.0. The elution and regeneration liquids were collected manually and the pools were analyzed by SDS-PAGE on 12.5 % Phast gels (Pharmacia) and silver staining. Prestained markers (Gibco) were included as relative molecular weight (Mr) standard. The major amount of proteins were retrieved in the flow through and an IGS5-candidate
20 band with a Mr of about 85.000 was observed in elution pools 1-3 (Fig.8). Western blot analysis of the lentil chromatography with the anti-His tag mab showed that the soluble hIGS5 protein (Mr ~ 85 000) is quantitatively bound to the Lentil Lectin resin and that the his-tagged protein is recovered over the whole elution peak, but mainly in pools 1 and 2 (Fig 9). The Lentil lectin elution pools 1 and 2 were further processed on the Zinc- IMAC
25 column (runs A and B).

Immobilized metal affinity chromatography (IMAC) and dialysis. 1 ml Chelating HiTrap (Pharmacia) was loaded with zinc ions as described by the manufacturer and equilibrated with buffer B (20 mM Hepes, 100 mM NaCl, 5% glycerol, 0.005% (w/v)
30 Tween 20, pH 7.2). Lentil elution pools 1 and 2 were loaded separately at 0.5 ml/min on the HiTrap column (IMAC run A and IMAC run B). A blank run was included to compare the chromatographic absorbance profile. The column was washed with buffer B till baseline level and bound proteins were eluted by applying an imidazole step gradient (20, 50, 100 and 200 mM) in buffer B. Fractions were collected manually. The IMAC column

was regenerated by applying 20 mM Hepes, 50 mM EDTA, 500 mM NaCl, pH 7.2. Elution and regeneration pools were analyzed by SDS-PAGE (12.5% Phast gels, Pharmacia) and silver staining. The 200 mM imidazole pool was transferred to a slide analyzer- cassette (MWCO 10.000, Pierce) and dialyzed overnight against buffer B (130 fold excess, no buffer refreshment). The amount of soluble IGS5 in the dialyzed pool was determined with the micro-BCA method (Pierce). BSA was included as reference. The dialyzed baculo IGS5 was biochemically characterized by (1) SDS-PAGE under reducing and non reducing conditions and (2) Western blot with an anti His-tag mAb (21E1B4, Innogenetics) followed by incubation with alkaline phosphatase labeled rabbit anti-mouse Ig (Dako) and detection with NBT/ BCIP staining. The glycosilation status of the soluble IGS5 was verified by PGNase F treatment (Biorad).

SDS-PAGE analysis and silver staining showed that the bulk of contaminating proteins were eluted by applying the 20 mM and 50 mM imidazole step (Fig. 10). The hIGS5 protein was retrieved in the 100 mM and 200 mM imidazole elution steps. The 85 kDa band in the 200 mM imidazole pool is a single band on the SDS-PAGE, which reacts with the anti his-tag mAb (Fig. 9). Silver staining did not reveal any difference in purity between the hIGS5 material obtained from IMAC run A and run B. Starting from 300 ml of baculo CM, 340 µg of over 95% pure his-tagged hIGS5 ectodomain was obtained by the 2 step purification procedure (i.e. a yield of about 1 mg/L).

The 200 mM imidazole Zn-IMAC pool was after SDS-PAGE blotted on PVDF membrane and the proteins were visualised by amido black staining. The PVDF bands were successively washed with 20% acetonitrile, and 20% methanol and dried. Amino-terminal sequencing was performed by Edman degradation using a Procise™ 492A (Applied biosystems) according to the manufacturer's description. Amino terminal sequencing confirmed that the Baculo IGS5 is recovered in the 85 kDa protein band.

EXAMPLE 7. ENZYME INHIBITION ASSAY.

The enzymatic activity of IGS5 polypeptides of the invention was tested with regard to the metabolism of biologically active peptides. In particular it was tested whether these IGS5 polypeptides may act on a variety of vasoactive peptides known in the state of the art e.g. such like atrial natriuretic peptide (ANP), bradykinin, big endothelin (Big ET-1), endothelin (ET-1), substance P and angiotensin-1. In the context of the present invention in

particular it was tested whether the IGS5 ectodomain, which is a novel human metalloprotease, hydrolyzes said vasoactive peptides. For comparison the assay was also performed for a known member of the metalloprotease family which was described earlier as soluble secreted endopeptidase (SEP) by Emoto et al. (J. Biol. Chem., Vol. 274 (1999): pp. 32469-32477). Furthermore, it was tested whether the activity of IGS5 to convert a Big-ET-1 analogue (the so-called 17 aa Big-ET) may be inhibited by reference compounds that are used to determine the inhibition properties with regard to enzymes having ECE and/or NEP-characteristics. Compounds used to test the inhibition of IGS5-activity on the Big-ET-1 analogue were the compound phosphoramidon which inhibits endopeptidases like NEP and ECE, the compound thiorphan which specifically inhibits NEP, and the compound CGS-35066 which is a selective ECE inhibitor.

Example 7a. Materials.

15

Enzyme: IGS-5 (sol hu)(his)6; or: His6-tagged IGS5 ectodomain;
stock solution: 53 mg/ml in 20 mM HEPES pH 7.2, 5% glycerol, 0.005% Tween20, 100 mM NaCl, purity >99%; storage at 4 °C.
working solution: stock solution diluted with assay buffer to 10 mg/ml.

20

Substrate: Mca-Asp-Ile-Ala-Trp-Phe-Dpa-Thr-Pro-Glu-His-Val-Val-Pro-Tyr-Gly-Leu-Gly-COOH;

Fluorescence-quenched Big-ET-1 analogue;

Mca = (7-Methoxycoumarin-4-yl);

25

Dpa = (3-[2,4-Dinitrophenyl]-L-2,3-diaminopropionyl);

stock solution: 100 µM in assay buffer; storage at -20 °C.

(commercially available from supplier: Polypeptide Laboratories, Wolfenbüttel, Germany)

30

Assay buffer: 100 mM Tris pH 7.0, 250 mM NaCl.

All test compounds were dissolved in DMSO at 10 mM and were further diluted with assay buffer.

Example 7b. Assay Procedure.

- 5 A quantity of 70 μ l of the assay buffer, of 10 μ l enzyme working solution and of 10 μ l test compound solution were mixed in an Eppendorf vial and preincubated at 37 °C for 15 minutes. Then, 10 μ l substrate stock solution was added and the reaction mixture was incubated at 37 °C for 60 minutes to allow for enzymatic hydrolysis. Subsequently the enzymatic reaction was terminated by heating at 95 °C for 5 minutes. After centrifugation
10 (Heraeus Biofuge B, 3 min) the supernatant was subjected to HPLC analysis.

Example 7c. HPLC Procedure.

- 15 In order to separate the remaining substrate from the cleavage products reversed phase HPLC technique was used with a CC 125/4 Nucleosil 300/5 C₁₈ RP column and a CC 8/4 Nucleosil 100/5 C₁₈ precolumn (commercially available from Macherey-Nagel, Düren, Germany). Thus, 60 μ l of the reaction samples obtained in Example 7b were injected into the HPLC, and the column was eluted at a flow rate of 1 ml/min by applying the following
20 gradient and solutions:

Solution A: 100% H₂O + 0.5 M H₃PO₄, pH = 2.0

Solution B: 100% acetonitrile + 0.5M H₃PO₄

25

0-2 min 20% B

2-6 min 20-60% B

6-8 min 60% B

8-10 min 60-90% B

30

10-13 min 90% B

13-15 min 90-100% B

Peptides were detected by absorbance at 214 nm and by fluorescence with an excitation wavelength of 328 nm and an emission wavelength of 393 nm.

Example 7d. Calculations.

5 The increasing fluorescence signal of the HPLC-peak of the peptide with the unquenched Mca-fluorophor after hydrolysis was taken as the basis for any calculation. This signal was compared for the samples with and without inhibitor and % inhibition was calculated on basis of the respective peak areas.

10 $\% \text{ inhib} = 100 \cdot (1 - A_{\text{inhib}} / A_{\text{control}})$

All samples were run in duplicate and mean values were used.

A standard inhibitor (10 nM and 100 nM Phosphoramidon) and a solvent control (0.1%)
15 was added to each assay run.

Example 7e. Results.

20 With regard to the IGS5 polypeptides of the present invention the results of Example 7 show that these IGS5 metalloprotease polypeptides hydrolyze in vitro a variety of vasoactive peptides known in the state of the art, in particular such as Big ET-1, ET-1, ANP and bradykinin. The results of the hydrolysis assay in comparison to the activity of SEP are shown in Table 10. From these results it is concluded that IGS5 may be
25 particularly involved in the metabolism of said biologically active peptides.

30 **Table 10: Hydrolysis of vasoactive peptides by IGS5 polypeptides in comparison to SEP (soluble secreted endopeptidase).**

Vasoactive Peptide	% Hydrolysis by IGS5 Polypeptide	% Hydrolysis by SEP (Emoto et al.)
	Conditions: 100 µg IGS5 polypeptide; 0.5 µM substrate; 2 h, 37 °C.	Conditions: 10 µg SEP; 0.5 µM substrate; 12 h, 37 °C.

ANP	5 (80*)	> 95
Bradykinin	100 (62**)	> 95
Big ET-1	(?)***	42
ET-1	30	92
Substance P	n.d.	> 95
Angiotensin 1	n.d.	> 95
17 aa Big ET	41	n.d.

* 500 µg IGS5 polypeptide

** 10 µg IGS5 polypeptide

*** activity was detected, but could not be quantified due to problems with the HPLC-detection

5

Furthermore, the results of the experiments with reference compounds for inhibition of ECE- and/or NEP-activity show that the activity of IGS5 metalloprotease polypeptides of the present invention to convert the Big-ET-1 analogue 17 aa Big-ET is inhibited by phosphoramidon, a reference compound for ECE/NEP-inhibition, but IGS5 is not efficiently inhibited by the NEP-inhibitor thiorphan. These results are shown in Table 11. IGS5 polypeptides are also not inhibited by the selective ECE-inhibitor CGS-35066, a potent and selective non-peptidic inhibitor of endothelin-converting enzyme-1 with sustained duration of action. (De Lombart et al., J. Med. Chem. 2000, Feb. 10; 43(3):488-504).

15

Table 11: Inhibition of IGS5 polypeptide's activity to convert the Big-ET-1 analogue 17 aa Big-ET.

20

Inhibitor Compound	IC ₅₀ nM
Phosphoramidon	18
Thiorphan	> 1000
CGS-35066	1300

PAGE INTENTIONALLY LEFT BLANK

Description of the figures:

Fig.1 Schematic representation of the relative positions of the different cDNA clones that were isolated and fully sequenced to generate the partial IGS5 consensus cDNA sequence. PCR primers that were used for 5' RACE and semi-homology PCR cloning are indicated and have been described in this document (indicated by the respective IP#). IGS5CONS denotes the consensus contig that was obtained after merging all obtained sequences. The 691 amino acids long open reading frame present in the IGS5 contig, that is postulated to contain the ectodomain of the IGS5 enzyme (IGS5DNA, IGS5PROT) is indicated with open boxes ("□□"). The part of the aligned EST sequences (accession no AA524283, AI088893, AI217369 and AI380811) that bears homology to members of the NEP/ECE family is indicated with "+==+"(IGS5EST). "bp" = base pairs.

Fig. 2 Schematic representation of the relative positions of the different cDNA clones that were isolated and fully sequenced to generate the IGS5DNA1 and IGS5DNA2 cDNA sequences. PCR primers that were used for PCR, 5' RACE and semi-homology PCR cloning are indicated and have been described in this document (indicated by the respective IP#). IGS5CONS1 and IGS5CONS2 denote the 2 different consensus contigs that were obtained after merging all obtained sequences. IGS5DNA1 and IGS5DNA2 denote the open reading frames present in IGS5CONS1 and IGS5CONS2 respectively ("***"). The part of the aligned EST sequences (accession n° AA524283, AI088893, AI217369 and AI 380811) that bears homology to members of the NEP/ECE family is indicated with "+==+" (IGS5EST). "bp" = base pairs. The 78 bp fragment identified within genomic clone IGS5/S1 is denoted as "IGS5/S1/78bp". The absence of the 78 bp alternate exon sequence within clones YCE231, YCE233 and YCE235 and within IGS5CONS2 and IGS5DNA2 is indicated by a gap.

Fig. 3 RNA Master Blot™ analysis of the IGS5 gene.

Fig. 4 Sequence of the 180 bp fragment, encoding the POMC signal sequence, the Gly-Ser linker, the His6 tag and the start of the IGS5 ectodomain sequence,

assembled by overlap PCR using different oligonucleotides. (*silent mutation (bp 57 of the pomc signal sequence)).

Fig. 5 Plasmid map of vector pAcSG2SOLhulGS5His6.

5

Fig. 6 Predicted protein sequence of the mature recombinant soluble His-tagged human IGS5, as expressed in Sf9 cells upon infection with recombinant baculovirus IGBV73 (after cleavage of the 26AA long POMC signal sequence). Potential N-glycosylation sites are underlined.

10

Fig. 7 Deglycosylation study – Western blot analysis. 72h CM harvest of the infection with the 3 recombinant soluble His6IGS5 clones (clone 1: lanes 1 to 3, clone2: lanes 4 to 6, clone 3: lanes 7 to 9) was treated as described with and without addition of N-glycosidase F. 10µl CM equivalent was loaded on gel versus 20µl of the non-treated CM as a control.

15

Detection was performed with anti-His antibody (21E1B4EPR300, Innogenetics, 1 µg/ml final concentration). Second antibody was rabbit anti mouse-Alkaline Phosphatase conjugated (Sigma A-1902). Revelation of the bands was done with NBT-BCIP. Mr marker is the Biolabs broad range MW marker (catn°7707S).

20

Fig. 8 SDS PAGE analysis under reducing conditions (+DTT) on 12.5% PHASTgel (Pharmacia; 4 µl/slot) of the Lentil chromatography steps. Proteins were visualised by silver staining.

25 Fig. 9 Western blot analysis of IGS5 at different stages of the purification procedure. Samples were separated on a 7.5% Minigel (Biorad MINI-Protean II) and analyzed via Western blot using the anti His6 primary mab 21E1B4, followed by an alkaline phosphatase conjugated rabbit anti mouse Ig as a secondary antibody and detection by NBT/BCIP.

30

+, - DTT resp. : proteins were reduced or not with DTT .

Fig.10 SDS PAGE analysis under reducing conditions (+DTT) on 12.5% PHASTgel (Pharmacia, 4µl slots) of different imidazole elution pools of the Zn-IMAC

chromatography of pool 1 from the lentil chromatography eluate. Proteins were visualised by silver staining.

Claims

1. An isolated polypeptide comprising an amino acid sequence which has at least 70% identity to one of the amino acid sequences selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6 over the entire length of the respective SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6.

2. An isolated polypeptide as claimed in claim 1 in which the amino acid sequence has at least 95% identity to one of the amino acid sequences selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6 over the entire length of the respective SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6.

3. An isolated polypeptide as claimed in claim 1 comprising one of the amino acid sequences selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6.

4. The isolated polypeptide of one of SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6, or a fragment thereof.

5. An isolated fragment of any of the isolated polypeptides as claimed in any one of claims 1 to 4 showing at least one of the characteristic activities of metalloproteases or structurally or functionally related enzymes.

6. An isolated polypeptide of any of the claims 1 to 4 or a polypeptide fragment of claim 5, which is a human polypeptide or human polypeptide fragment.

7. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 70% identity to one of the amino acid sequences selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, over the entire length of the respective SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6, or a nucleotide sequence complementary to said isolated polynucleotide.

8. An isolated polynucleotide comprising a nucleotide sequence that has at least 70% identity to a nucleotide sequence encoding a polypeptide selected from the group of

SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, over the entire coding region; or a nucleotide sequence complementary to said isolated polynucleotide.

9. An isolated polynucleotide comprising a nucleotide sequence which has at
5 least 70% identity to one of that selected from the group of SEQ ID NO:1, SEQ ID NO:3
and SEQ ID NO:5, over the entire length of the respective SEQ ID NO:1, SEQ ID NO:3 or
SEQ ID NO:5; or a nucleotide sequence complementary to said isolated polynucleotide.

10. The isolated polynucleotide as claimed in any one of claims 7 to 9 in which the
10 identity is at least 95%.

11. An isolated polynucleotide selected from the group of:

- (a) a polynucleotide comprising a nucleotide sequence encoding one of the polypeptides
selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6;
- 15 (b) a polynucleotide selected from the group of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID
NO:5; and
- (c) a polynucleotide obtainable by screening an appropriate library under stringent
hybridization conditions with a labeled probe having the sequence selected from the
group of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, or of a fragment thereof;
- 20 or a nucleotide sequence complementary to said isolated polynucleotide.

12. An isolated polynucleotide fragment encoding a polypeptide fragment as
defined in claim 5, or a nucleotide sequence complementary to said isolated
polynucleotide fragment.

25

13. The polynucleotide of any of claims 7 to 12 which is DNA or RNA.

14. The polynucleotide of any of claims 7 to 13 which is a human polynucleotide.

30 15. An expression system comprising a polynucleotide capable of producing a
polypeptide of any of claims 1 to 6, when said expression system is present in a
compatible host cell.

16. A host cell comprising the expression system of claim 15 or a membrane thereof expressing the polypeptide of any of claims 1 to 6.

17. A process for producing a polypeptide of any of claims 1 to 6 comprising
5 culturing a host cell of claim 16 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

18. An antibody immunospecific for the polypeptide of any of claims 1 to 4, for a polypeptide fragment of claim 5, or for a polypeptide of claim 6.

10

19. A method for screening to identify compounds which influence the activity of the polypeptide of any one of claims 1 to 4, or of the polypeptide fragment of claim 5, or of the polypeptide of claim 6, comprising:

- 15 (a) contacting cells or membranes of claim 16 bearing a polypeptide of claims 1 to 6, or contacting a polypeptide of claims 1 to 4, a fragment thereof according to claim 5, or a polypeptide of claim 6 with a candidate compound; and
- (b) assessing whether said candidate compound results in a stimulation or inhibition of the activity of said polypeptide.

20. A method for screening to identify compounds which stimulate or which inhibit the function of the polypeptide of any one of claims 1 to 4, or of the polypeptide fragment of claim 5, or of the polypeptide of claim 6, which comprises a method selected from the group consisting of:

- 25 (a) measuring the influence of a candidate compound on the activity of the polypeptide, or on the cells or membranes bearing the polypeptide, or a fusion protein thereof in the presence of a suitable substrate for said polypeptide;
- (b) measuring the influence of a candidate compound on the activity of the polypeptide, or on the cells or membranes bearing the polypeptide, or a fusion protein thereof in the presence of a competitor of said polypeptide;
- 30 (c) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the activity of the polypeptide or to the cells or membranes bearing the polypeptide;
- (d) mixing a candidate compound with a solution containing a polypeptide of claim 1 and a suitable substrate, to form a mixture, measuring activity of the polypeptide in the

mixture, and comparing the activity of the mixture to a standard without candidate compound; or

- (e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide and said polypeptide in cells, using for instance, an ELISA assay.

5

21. A method according to claim 19 or 20 in which the screening serves to identify compounds suitable for the treatment and/or prophylaxis of cardiovascular diseases.

22. A stimulant or inhibitor to the polypeptide of claims 1 to 4, to a polypeptide fragment of claim 5, or to a polypeptide of claim 6, identified by any method of any of the claims 19 to 21.

23. A compound for use in therapy, which is selected from the group of:

(a) a stimulant or inhibitor to the polypeptide of any of claims 1 to 4 and 6, identified by any method of any of the claims 19 to 21;

(b) an stimulant or inhibitor to the polypeptide fragment of claim 5 or 6, identified by any method of any of the claims 19 to 21;

(c) an isolated polynucleotide of claims 7 to 14; or

(d) a nucleic acid molecule that modulates the expression of the nucleotide sequence encoding the polypeptide of claim 1.

24. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of the polypeptide of claim 1 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said polypeptide in the genome of said subject; and/or

(a) analyzing for the presence or amount of said polypeptide expression in a sample derived from said subject.

25. A method for the treatment of a subject in need of enhanced IGS5 activity comprising:

(a) administering to the subject a therapeutically effective amount of a stimulator to said IGS5; and/or

(b) providing to the subject the IGS5 polynucleotide in a form so as to effect production of said IGS5 activity in vivo.

26. A method for the treatment of a subject having need of decreased IGS5 activity comprising:

- 5 (a) administering to the subject a therapeutically effective amount of an inhibitor to said IGS5, the inhibitor being identified by any method of any of the claims 19 to 21; and/or
- (b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said IGS5; and/or
- (c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said IGS5 for its substrate; and/or
- 10 (d) administering to the subject a therapeutically effective amount of a polypeptide that degrades said IGS5; and/or
- (e) administering to the subject a nucleic acid molecule that enhances the expression of a nucleotide sequence encoding for a polypeptide that degrades said IGS5.

- 15 27. A stimulant or inhibitor according to claim 22 or a compound for use in therapy according to claim 23, for the treatment and/or prophylaxis of cardiovascular diseases.

- 28. A process for diagnosing according to claim 24 or method of treatment according to claim 25 or 26, in which the disease or the enhanced or decreased IGS5
20 activity is linked to a cardiovascular disease.

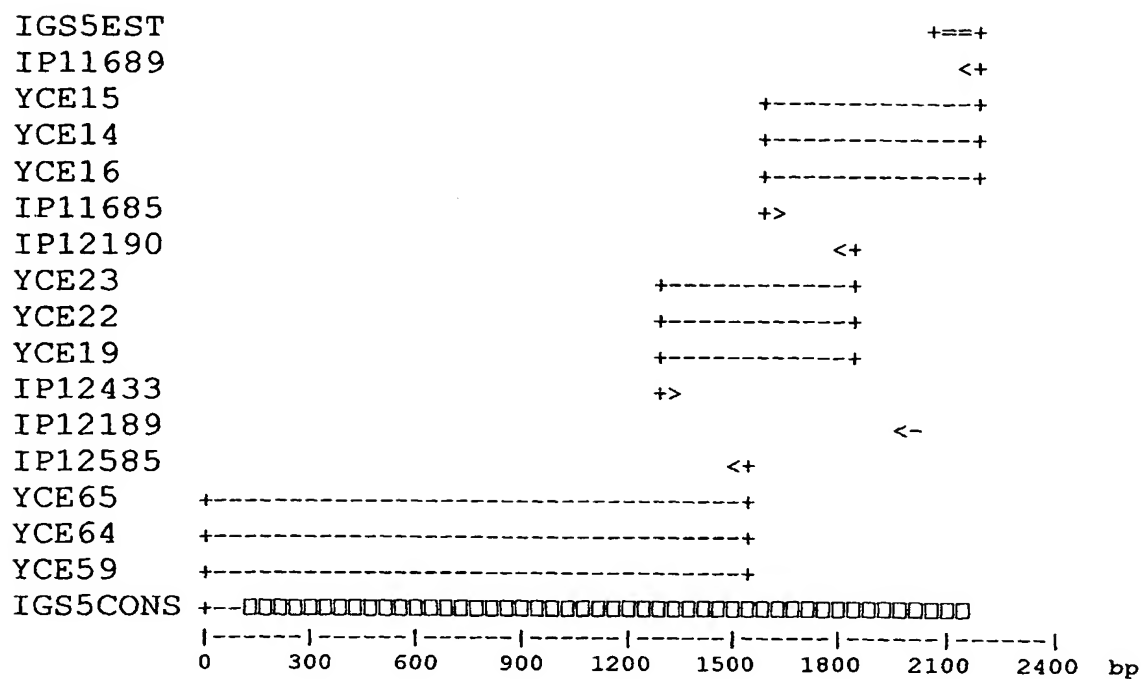


FIG.1.

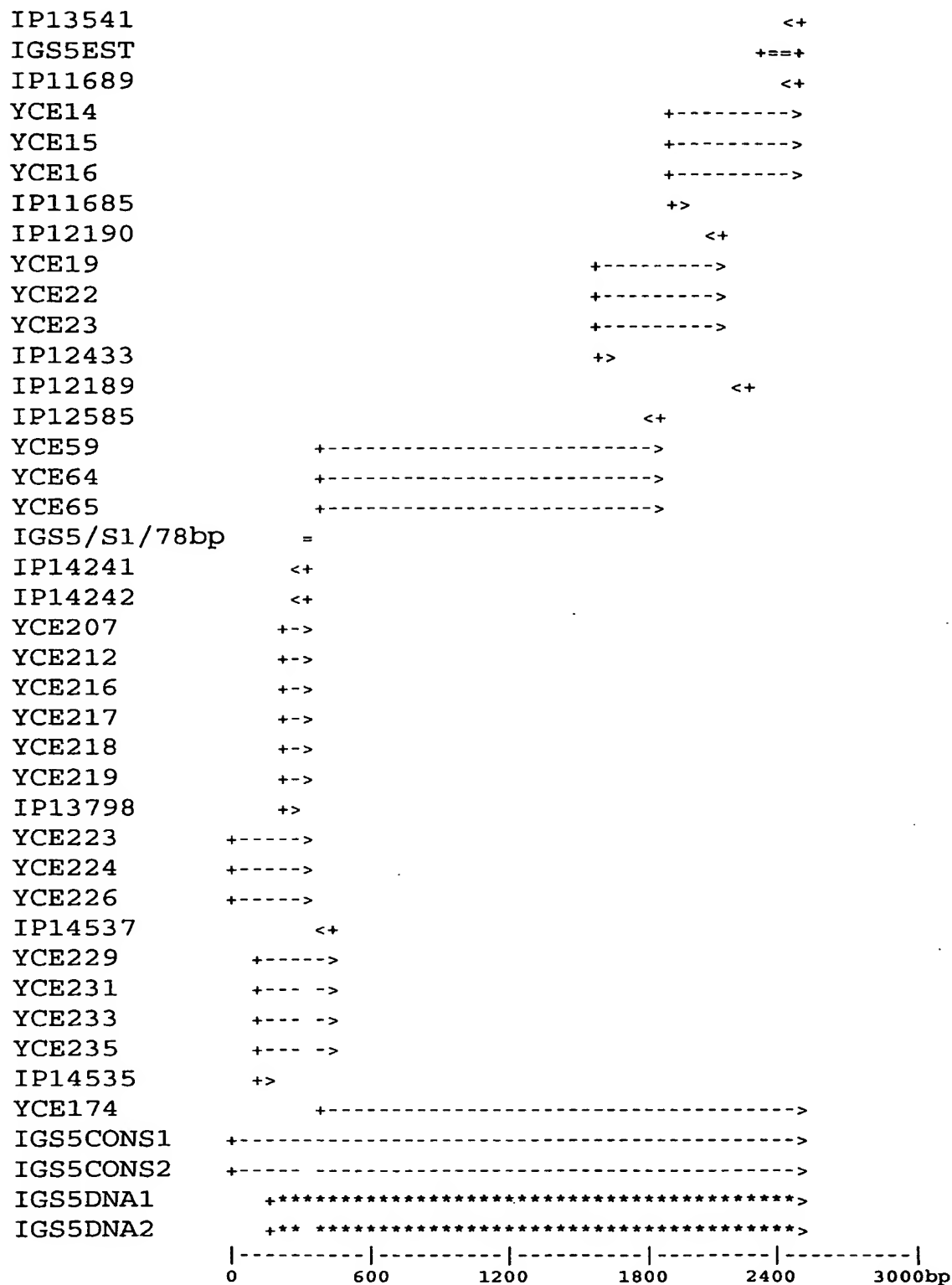


FIG.2.

	1	2	3	4	5	6	7	8
A	whole brain	amygdalia	caudate nucleus	cerebellum	cerebral cortex	frontal lobe	hippocampus	medulla oblongata
B	occipital lobe	putamen	substantia nigra	temporal lobe	thalamus	nucleus accumbens	spinal cord	
C	heart	aorta	skeletal muscle	colon	bladder	uterus	prostate	stomach
D	testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
E	kidney	liver	small intestine	spleen	thymus	peripheral leukocyte	lymph node	bone marrow
F	appendix	lung	trachea	placenta				
G	fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	
H	yeast total RNA 100ng	yeast tRNA 100ng	E.coli rRNA 100ng	E.coli tRNA 100ng	Poly (A) 100ng	human 6,7-1 DNA 100ng	human DNA 100ng	human DNA 500ng

BEST AVAILABLE COPY

FIG. 3
SUBSTITUTE SHEET (RULE 26)

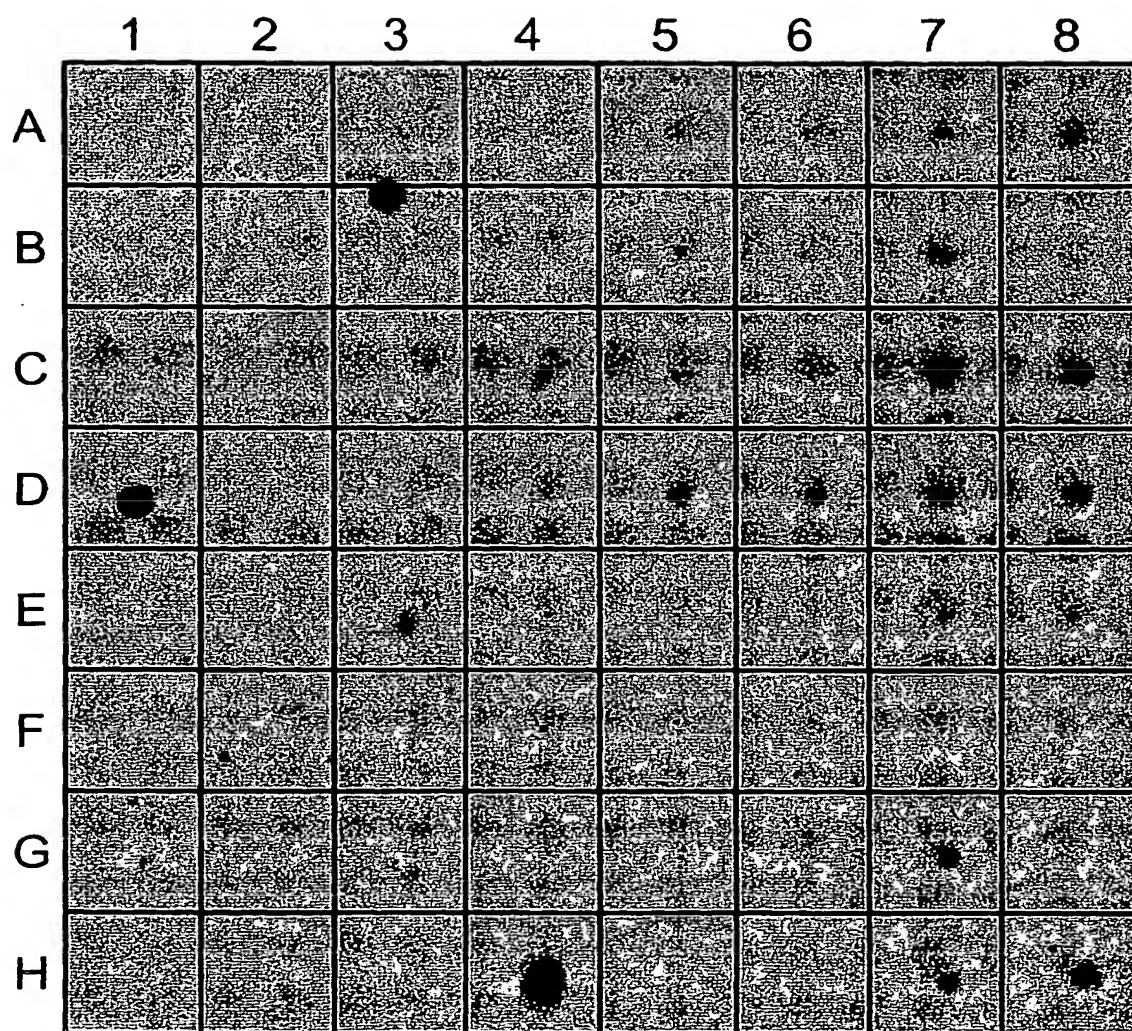


Fig. 3

SUBSTITUTE SHEET (RULE 26)

StuI **start** POMC SIGNAL SEQUENCE
GACAAGGCCT ATT**ATG**CCGA GATCGTGCTG CAGCCGCTCG
CTGTTCCGGA TAATACGGCT CTAGCACGAC GTCGGCGAGC

GGGGCCCTGT TGCTGGCCTT GCTGCTTCAA* GCCTCCATGG
CCCCGGGACA ACGACCGGAA CGACGAAGTT CGGAGGTACC

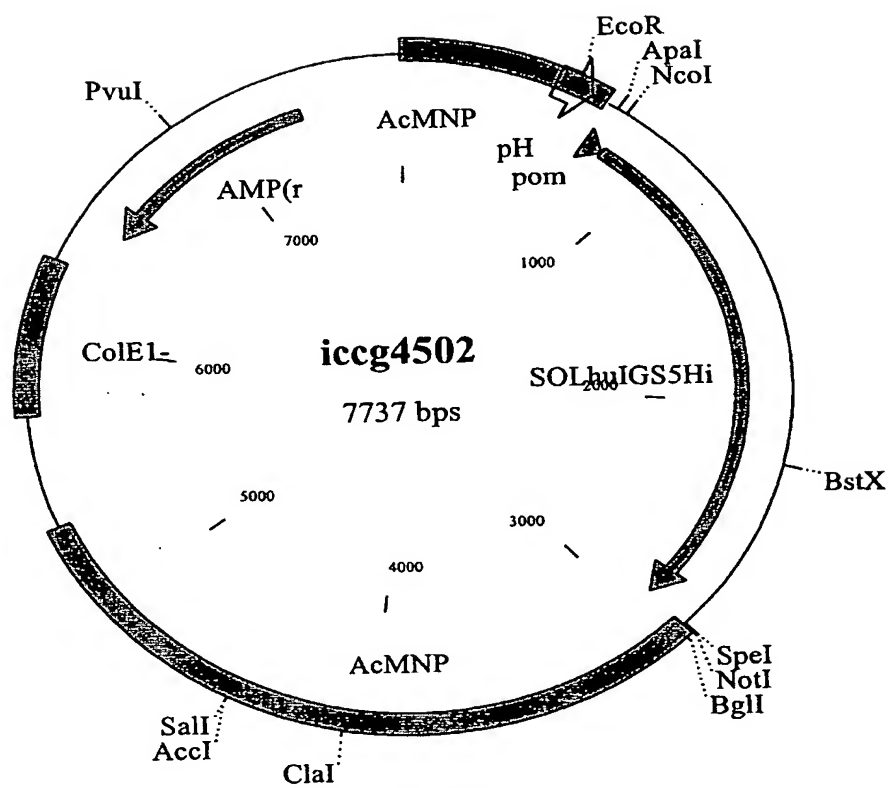
 GS linker His6
AAGTGCGTGG CGGTTCTCAC CATCACCACC ATCACAGCGA
TTCACGCACC GCCAAGAGTG GTAGTGGTGG TAGTGTCGCT

GGTCTGCACC ACCCCTGGCT GCGTGATAGC AGCTGCCAGG
CCAGACGTGG TGGGGACCGA CGCACTATCG TCGACGGTCC

ATCCTCCAGA ACATGGACCC
TAGGAGGTCT TGTACCTGGG
HuIGS5 overlapping sequence

FIG.4.

FIG.5.



GSHHHHHHSEVCTTPGCVIAAARILQNMDPTTEPCDDFYQFACGGWLRRHVIPETNS
RYSIFDVLRLDELEVILKAVLE~~N~~STAKDRPAVEKARTLYRSCM~~N~~OSVLEKRGSQPLLD
ILEVVGGWPVAMDRW~~N~~ETVGLEWELERQLALMNSQFNRRVLIDLFIW~~N~~DDO~~N~~SSRHI
IYIDQPTLGMPsREYYFNNGSNRKVREAYLQFMVSVATLLREDANLPRDSCLVQEDM
MQVLELETQLAKATVPQEERHDVIALYHRMGLEELQSQFGLKGF~~N~~WTLEIQTVLSSV
KIKLLPDEEVVVYGI PYLQNLNII DTYSARTIQNYLVWRLVLD RIGSLSQRFKDTR
VNYRKALFGTMVEEVRWRECVGYVNSNMENAVGSLYVREAFPGDSKSMVRELIDKVR
TVFVETLDELGWMDEESKKKAQEKAMSIREQIGHPDYILEEMNRRLDEEYSNL~~N~~FSE
DL~~Y~~FENSLQNLKVGAQRSLRKLREKVDPNLWII GAAVVNAFYSPNRNQIVFPAGILQ
PPFFSKEQPQALNFGGIGMVGHEITHGFDDNGRNF~~D~~KNGNM~~M~~DWWS~~N~~FSTO~~H~~FREQ
SECM~~I~~YOYGN~~Y~~SWDLADEQNVNGFNTLGENIADNGGVRQAYKAYLKWMAEGGKDQQL
PGLDLTHEQLFFINYAQVWCGSYRPEFAIQSIKTDVHSPLKYRVLGSLQNLAA~~F~~ADT
FHCARGTPMHPKERCRVW

FIG.6.

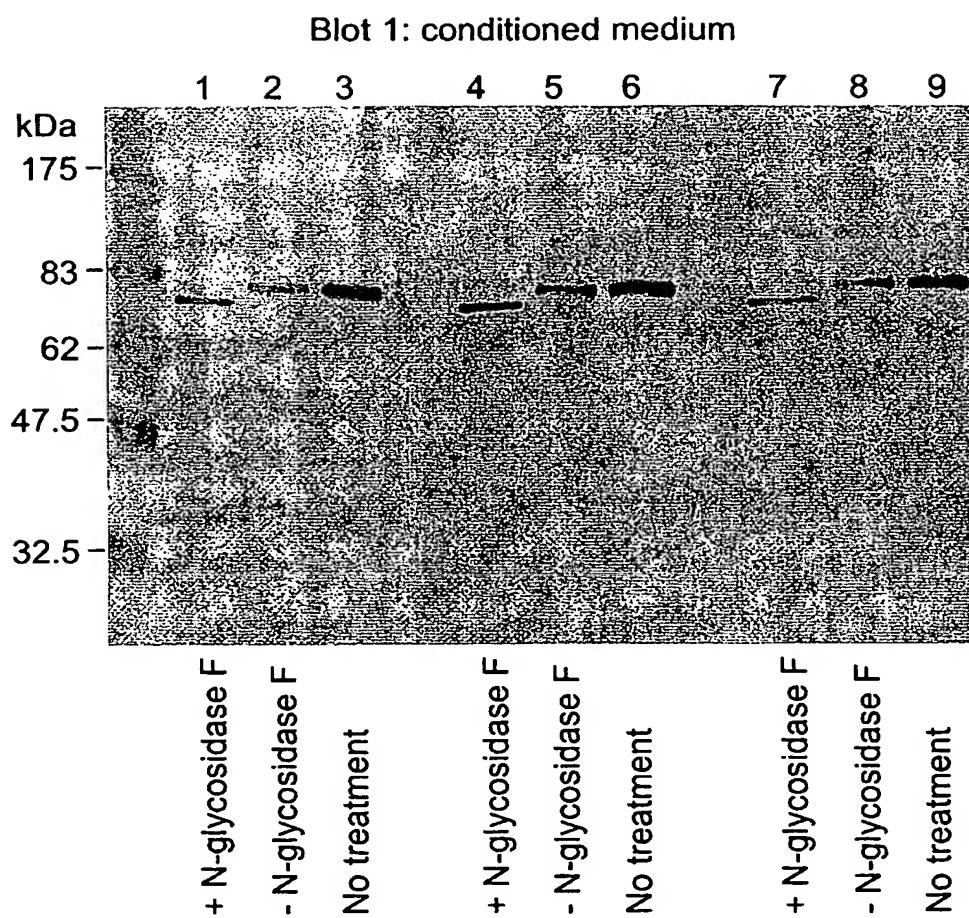
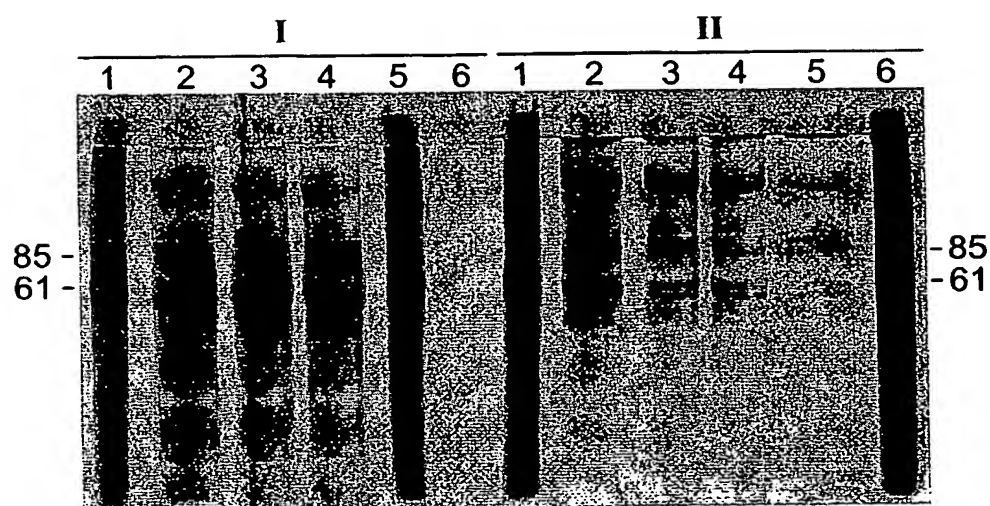
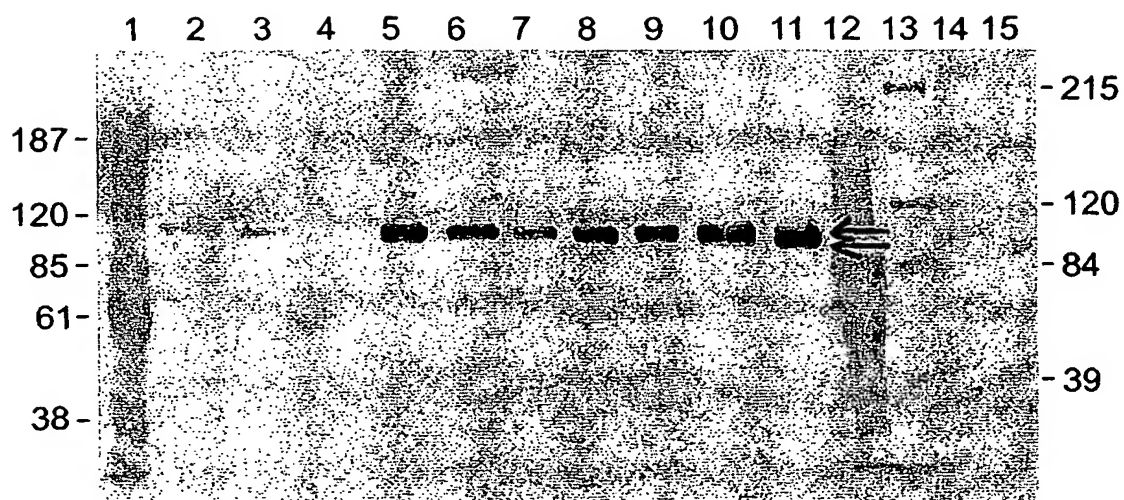


FIG. 7



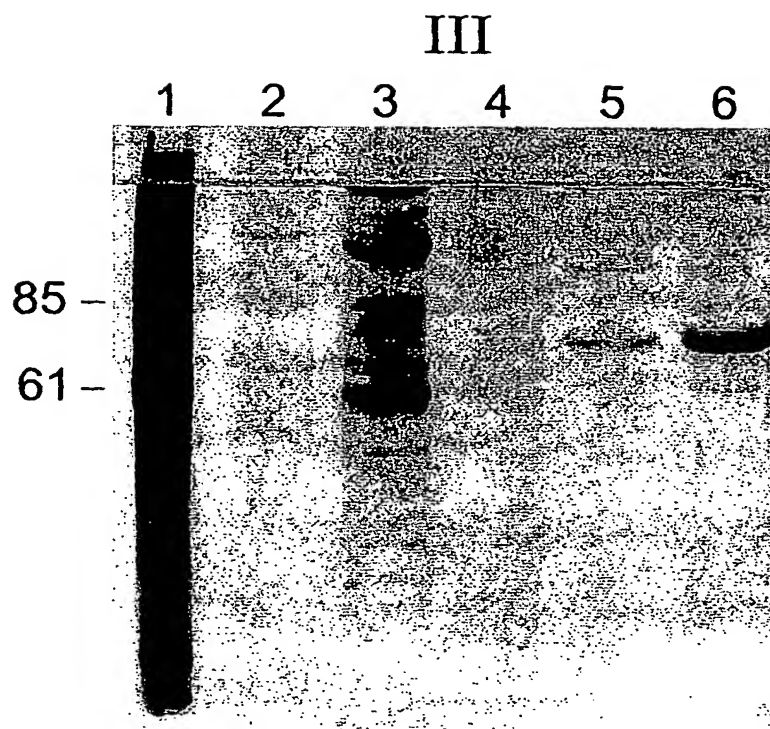
Lane	Sample	DTT
I.1	Prestained Marker	+
2	Start before pretreatment	+
3	Start after pretreatment	+
4	Flow through	+
5	Prestained Marker	+
6	/	
II.1	Prestained Marker	+
2	Pool 1 500mM MMP-eluate	+
3	Pool 2 "	+
4	Pool 3 "	+
5	Pool 4 "	+
6	Prestained Marker	+

FIG. 8



Lane	Sample	DTT
1	Prestained Marker (Gibco)	+
2	Start crude	+
3	Start Lentil	+
4	Flow through Lentil	+
5	500mM MMP Lentil-eluate (Pool 1)	+
6	500mM MMP Lentil-eluate (Pool 2)	+
7	500mM MMP Lentil-eluate (Pool 3)	+
8	200mM imidazole IMAC-eluate (< Pool 1)	+
9	200mM imidazole IMAC-eluate (< Pool 2)	+
10	200mM pool after dialysis	+
11	200mM pool after dialysis	-
12	Prestained Marker (Gibco)	+
13	Prestained Marker (Pierce)	+

FIG. 9



Lane	Sample	DTT
III.1	Prestained Marker	+
2	Flow through	+
3	20mM Pool	+
4	50mM Pool	+
5	100mM Pool	+
6	200mM Pool	+

FIG. 10
SUBSTITUTE SHEET (RULE 26)

SEQUENCE LISTING

<110> SOLVAY PHARMACEUTICALS B.V.

<120> Novel Human Enzymes of the Metalloprotease Family

<130> SPW 99.09 /H 99.26-WO

<140>

<141>

<160> 31

<170> PatentIn Ver. 2.1

<210> 1

<211> 2076

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1) .. (2073)

<400> 1

tgc acc acc cct ggc tgc gtg ata gca gct gcc agg atc ctc cag aac	48
Cys Thr Thr Pro Gly Cys Val Ile Ala Ala Ala Arg Ile Leu Gln Asn	
1 5 10 15	
atg gac ccg acc acg gaa ccg tgt gac gac ttc tac cag ttt gca tgc	96
Met Asp Pro Thr Thr Glu Pro Cys Asp Asp Phe Tyr Gln Phe Ala Cys	
20 25 30	
gga ggc tgg ctg cgg cgc cac gtg atc cct gag acc aac tca aga tac	144
Gly Gly Trp Leu Arg Arg His Val Ile Pro Glu Thr Asn Ser Arg Tyr	
35 40 45	
agc atc ttt gac gtc ctc cgc gac gag ctg gag gtc atc ctc aaa gcg	192
Ser Ile Phe Asp Val Leu Arg Asp Glu Leu Glu Val Ile Leu Lys Ala	
50 55 60	
gtg ctg gag aat tcg act gcc aag gac cgg ccg gct gtg gag aag gcc	240
Val Leu Glu Asn Ser Thr Ala Lys Asp Arg Pro Ala Val Glu Lys Ala	
65 70 75 80	
agg acg ctg tac cgc tcc tgc atg aac cag agt gtg ata gag aag cga	288
Arg Thr Leu Tyr Arg Ser Cys Met Asn Gln Ser Val Ile Glu Lys Arg	
85 90 95	
ggc tct cag ccc ctg ctg gac atc ttg gag gtg gtg gga ggc tgg ccg	336
Gly Ser Gln Pro Leu Leu Asp Ile Leu Glu Val Val Gly Gly Trp Pro	
100 105 110	
gtg gcg atg gac agg tgg aac gag acc gta gga ctc gag tgg gag ctg	384
Val Ala Met Asp Arg Trp Asn Glu Thr Val Gly Leu Glu Trp Glu Leu	
115 120 125	
gag cgg cag ctg gcg ctg atg aac tca cag ttc aac agg cgc gtc ctc	432
Glu Arg Gln Leu Ala Leu Met Asn Ser Gln Phe Asn Arg Arg Val Leu	
130 135 140	

atc gac ctc ttc atc tgg aac gac gac cag aac tcc agc cgg cac atc	480
Ile Asp Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser Ser Arg His Ile	
145 150 155 160	
atc tac ata gac cag ccc acc ttg ggc atg ccc tcc cga gag tac tac	528
Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser Arg Glu Tyr Tyr	
165 170 175	
ttc aac ggc ggc agc aac cgg aag gtg cgg gaa gcc tac ctg cag ttc	576
Phe Asn Gly Gly Ser Asn Arg Lys Val Arg Glu Ala Tyr Leu Gln Phe	
180 185 190	
atg gtg tca gtg gcc acg ttg ctg cgg gag gat gca aac ctg ccc agg	624
Met Val Ser Val Ala Thr Leu Arg Glu Asp Ala Asn Leu Pro Arg	
195 200 205	
gac agc tgc ctg gtg cag gag gac atg atg cag gtg ctg gag ctg gag	672
Asp Ser Cys Leu Val Gln Glu Asp Met Met Gln Val Leu Glu Leu Glu	
210 215 220	
aca cag ctg gcc aag gcc acg gta ccc cag gag gag aga cac gac gtc	720
Thr Gln Leu Ala Lys Ala Thr Val Pro Gln Glu Glu Arg His Asp Val	
225 230 235 240	
atc gcc ttg tac cac cgg atg gga ctg gag gag ctg caa agc cag ttt	768
Ile Ala Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln Ser Gln Phe	
245 250 255	
ggc ctg aag gga ttt aac tgg act ctg ttc ata caa act gtg cta tcc	816
Gly Leu Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Thr Val Leu Ser	
260 265 270	
tct gtc aaa atc aag ctg ctg cca gat gag gaa gtg gtg gtc tat ggc	864
Ser Val Lys Ile Lys Leu Leu Pro Asp Glu Glu Val Val Tyr Gly	
275 280 285	
atc ccc tac ctg cag aac ctt gaa aac atc atc gac acc tac tca gcc	912
Ile Pro Tyr Leu Gln Asn Leu Glu Asn Ile Ile Asp Thr Tyr Ser Ala	
290 295 300	
agg acc ata cag aac tac ctg gtc tgg cgc ctg gtg ctg gac cgc att	960
Arg Thr Ile Gln Asn Tyr Leu Val Trp Arg Leu Val Leu Asp Arg Ile	
305 310 315 320	
ggt agc cta agc cag aga ttc aag gac aca cga gtg aac tac cgc aag	1008
Gly Ser Leu Ser Gln Arg Phe Lys Asp Thr Arg Val Asn Tyr Arg Lys	
325 330 335	
gcg ctg ttt ggc aca atg gtg gag gag gtg cgc tgg cgt gaa tgt gtg	1056
Ala Leu Phe Gly Thr Met Val Glu Glu Val Arg Trp Arg Glu Cys Val	
340 345 350	
ggc tac gtc aac agc aac atg gag aac gcc gtg ggc tcc ctc tac gtc	1104
Gly Tyr Val Asn Ser Asn Met Glu Asn Ala Val Gly Ser Leu Tyr Val	
355 360 365	
agg gag gcg ttc cct gga gac agc aag agc atg gtc aga gaa ctc att	1152
Arg Glu Ala Phe Pro Gly Asp Ser Lys Ser Met Val Arg Glu Leu Ile	
370 375 380	
gac aag gtg cgg aca gtg ttt gtg gag acg ctg gac gag ctg ggc tgg	1200

Asp 385	Lys	Val	Arg	Thr	Val	Phe	Val	Glu	Thr	Leu	Asp	Glu	Leu	Gly	Trp	
					390					395					400	
atg	gac	gag	gag	tcc	aag	aag	aag	gcg	cag	gag	aag	gcc	atg	agc	atc	1248
Met	Asp	Glu	Glu	Ser	Lys	Lys	Lys	Ala	Gln	Glu	Lys	Ala	Met	Ser	Ile	
				405				410						415		
cgg	gag	cag	atc	ggg	cac	cct	gac	tac	atc	ctg	gag	gag	atg	aac	agg	1296
Arg	Glu	Gln	Ile	Gly	His	Pro	Asp	Tyr	Ile	Leu	Glu	Glu	Met	Asn	Arg	
			420					425						430		
cgc	ctg	gac	gag	gag	tac	tcc	aat	ctg	aac	ttc	tca	gag	gac	ctg	tac	1344
Arg	Leu	Asp	Glu	Glu	Tyr	Ser	Asn	Leu	Asn	Phe	Ser	Glu	Asp	Leu	Tyr	
		435					440					445				
ttt	gag	aac	agt	ctg	cag	aac	ctc	aag	gtg	ggc	gcc	cag	cgg	agc	ctc	1392
Phe	Glu	Asn	Ser	Leu	Gln	Asn	Leu	Lys	Val	Gly	Ala	Gln	Arg	Ser	Leu	
	450					455					460					
agg	aag	ctt	cgg	gaa	aag	gtg	gac	cca	aat	ctc	tgg	atc	atc	ggg	gcg	1440
Arg	Lys	Leu	Arg	Glu	Lys	Val	Asp	Pro	Asn	Leu	Trp	Ile	Ile	Gly	Ala	
	465				470					475					480	
gcg	gtg	gtc	aat	gcg	ttc	tac	tcc	cca	aac	cga	aac	cag	att	gta	ttc	1488
Ala	Val	Val	Asn	Ala	Phe	Tyr	Ser	Pro	Asn	Arg	Asn	Gln	Ile	Val	Phe	
				485					490					495		
cct	gcc	ggg	atc	ctc	cag	ccc	ccc	ttc	ttc	agc	aag	gag	cag	cca	cag	1536
Pro	Ala	Gly	Ile	Leu	Gln	Pro	Pro	Phe	Phe	Ser	Lys	Glu	Gln	Pro	Gln	
			500					505					510			
gcc	ttg	aac	ttt	gga	ggc	att	ggg	atg	gtg	atc	ggg	cac	gag	atc	acg	1584
Ala	Leu	Asn	Phe	Gly	Gly	Ile	Gly	Met	Val	Ile	Gly	His	Glu	Ile	Thr	
		515					520					525				
cac	ggc	ttt	gac	gac	aat	ggc	cgg	aac	ttc	gac	aag	aat	ggc	aac	atg	1632
His	Gly	Phe	Asp	Asp	Asn	Gly	Arg	Asn	Phe	Asp	Lys	Asn	Gly	Asn	Met	
	530					535					540					
atg	gat	tgg	tgg	agt	aac	ttc	tcc	acc	cag	cac	ttc	cgg	gag	cag	tca	1680
Met	Asp	Trp	Trp	Ser	Asn	Phe	Ser	Thr	Gln	His	Phe	Arg	Glu	Gln	Ser	
	545				550					555					560	
gag	tgc	atg	atc	tac	cag	tac	ggc	aac	tac	tcc	tgg	gac	ctg	gca	gac	1728
Glu	Cys	Met	Ile	Tyr	Gln	Tyr	Gly	Asn	Tyr	Ser	Trp	Asp	Leu	Ala	Asp	
				565					570					575		
gaa	cag	aac	gtg	aac	gga	ttc	aac	acc	ctt	ggg	gaa	aac	att	gct	gac	1776
Glu	Gln	Asn	Val	Asn	Gly	Phe	Asn	Thr	Leu	Gly	Glu	Asn	Ile	Ala	Asp	
			580					585					590			
aac	gga	ggg	gtg	cgg	caa	gcc	tat	aag	gcc	tac	ctc	aag	tgg	atg	gca	1824
Asn	Gly	Gly	Val	Arg	Gln	Ala	Tyr	Lys	Ala	Tyr	Leu	Lys	Trp	Met	Ala	
		595					600					605				
gag	ggg	ggc	aag	gac	cag	cag	ctg	ccc	ggc	ctg	gat	ctc	acc	cat	gag	1872
Glu	Gly	Gly	Lys	Asp	Gln	Gln	Leu	Pro	Gly	Leu	Asp	Leu	Thr	His	Glu	
	610					615					620					
cag	ctc	ttc	ttc	atc	aac	tac	gcc	cag	gtg	tgg	tgc	ggg	tcc	tac	cgg	1920
Gln	Leu	Phe	Phe	Ile	Asn	Tyr	Ala	Gln	Val	Trp	Cys	Gly	Ser	Tyr	Arg	

```

625                630                635                640
ccc gag ttc gcc atc caa tcc atc aag aca gac gtc cac agt ccc ctg 1968
Pro Glu Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His Ser Pro Leu
                645                650                655

aag tac agg gta ctg ggg tcg ctg cag aac ctg gcc gcc ttc gca gac 2016
Lys Tyr Arg Val Leu Gly Ser Leu Gln Asn Leu Ala Ala Phe Ala Asp
                660                665                670

acg ttc cac tgt gcc cgg ggc acc ccc atg cac ccc aag gag cga tgc 2064
Thr Phe His Cys Ala Arg Gly Thr Pro Met His Pro Lys Glu Arg Cys
                675                680                685

cgc gtg tgg tag 2076
Arg Val Trp
        690

<210> 2
<211> 691
<212> PRT
<213> Homo sapiens

<400> 2
Cys Thr Thr Pro Gly Cys Val Ile Ala Ala Ala Arg Ile Leu Gln Asn
  1                5                10                15

Met Asp Pro Thr Thr Glu Pro Cys Asp Asp Phe Tyr Gln Phe Ala Cys
                20                25                30

Gly Gly Trp Leu Arg Arg His Val Ile Pro Glu Thr Asn Ser Arg Tyr
  35                40                45

Ser Ile Phe Asp Val Leu Arg Asp Glu Leu Glu Val Ile Leu Lys Ala
  50                55                60

Val Leu Glu Asn Ser Thr Ala Lys Asp Arg Pro Ala Val Glu Lys Ala
  65                70                75                80

Arg Thr Leu Tyr Arg Ser Cys Met Asn Gln Ser Val Ile Glu Lys Arg
                85                90                95

Gly Ser Gln Pro Leu Leu Asp Ile Leu Glu Val Val Gly Gly Trp Pro
  100                105                110

Val Ala Met Asp Arg Trp Asn Glu Thr Val Gly Leu Glu Trp Glu Leu
  115                120                125

Glu Arg Gln Leu Ala Leu Met Asn Ser Gln Phe Asn Arg Arg Val Leu
  130                135                140

Ile Asp Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser Ser Arg His Ile
  145                150                155                160

Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser Arg Glu Tyr Tyr
                165                170                175

Phe Asn Gly Gly Ser Asn Arg Lys Val Arg Glu Ala Tyr Leu Gln Phe
  180                185                190

```

Met Val Ser Val Ala Thr Leu Leu Arg Glu Asp Ala Asn Leu Pro Arg
 195 200 205
 Asp Ser Cys Leu Val Gln Glu Asp Met Met Gln Val Leu Glu Leu Glu
 210 215 220
 Thr Gln Leu Ala Lys Ala Thr Val Pro Gln Glu Glu Arg His Asp Val
 225 230 235 240
 Ile Ala Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln Ser Gln Phe
 245 250 255
 Gly Leu Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Thr Val Leu Ser
 260 265 270
 Ser Val Lys Ile Lys Leu Leu Pro Asp Glu Glu Val Val Val Tyr Gly
 275 280 285
 Ile Pro Tyr Leu Gln Asn Leu Glu Asn Ile Ile Asp Thr Tyr Ser Ala
 290 295 300
 Arg Thr Ile Gln Asn Tyr Leu Val Trp Arg Leu Val Leu Asp Arg Ile
 305 310 315 320
 Gly Ser Leu Ser Gln Arg Phe Lys Asp Thr Arg Val Asn Tyr Arg Lys
 325 330 335
 Ala Leu Phe Gly Thr Met Val Glu Glu Val Arg Trp Arg Glu Cys Val
 340 345 350
 Gly Tyr Val Asn Ser Asn Met Glu Asn Ala Val Gly Ser Leu Tyr Val
 355 360 365
 Arg Glu Ala Phe Pro Gly Asp Ser Lys Ser Met Val Arg Glu Leu Ile
 370 375 380
 Asp Lys Val Arg Thr Val Phe Val Glu Thr Leu Asp Glu Leu Gly Trp
 385 390 395 400
 Met Asp Glu Glu Ser Lys Lys Lys Ala Gln Glu Lys Ala Met Ser Ile
 405 410 415
 Arg Glu Gln Ile Gly His Pro Asp Tyr Ile Leu Glu Glu Met Asn Arg
 420 425 430
 Arg Leu Asp Glu Glu Tyr Ser Asn Leu Asn Phe Ser Glu Asp Leu Tyr
 435 440 445
 Phe Glu Asn Ser Leu Gln Asn Leu Lys Val Gly Ala Gln Arg Ser Leu
 450 455 460
 Arg Lys Leu Arg Glu Lys Val Asp Pro Asn Leu Trp Ile Ile Gly Ala
 465 470 475 480
 Ala Val Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Gln Ile Val Phe
 485 490 495
 Pro Ala Gly Ile Leu Gln Pro Pro Phe Phe Ser Lys Glu Gln Pro Gln
 500 505 510
 Ala Leu Asn Phe Gly Gly Ile Gly Met Val Ile Gly His Glu Ile Thr

515					520					525					
His	Gly	Phe	Asp	Asp	Asn	Gly	Arg	Asn	Phe	Asp	Lys	Asn	Gly	Asn	Met
530						535					540				
Met	Asp	Trp	Trp	Ser	Asn	Phe	Ser	Thr	Gln	His	Phe	Arg	Glu	Gln	Ser
545					550					555					560
Glu	Cys	Met	Ile	Tyr	Gln	Tyr	Gly	Asn	Tyr	Ser	Trp	Asp	Leu	Ala	Asp
				565					570						575
Glu	Gln	Asn	Val	Asn	Gly	Phe	Asn	Thr	Leu	Gly	Glu	Asn	Ile	Ala	Asp
			580					585					590		
Asn	Gly	Gly	Val	Arg	Gln	Ala	Tyr	Lys	Ala	Tyr	Leu	Lys	Trp	Met	Ala
		595					600					605			
Glu	Gly	Gly	Lys	Asp	Gln	Gln	Leu	Pro	Gly	Leu	Asp	Leu	Thr	His	Glu
	610					615					620				
Gln	Leu	Phe	Phe	Ile	Asn	Tyr	Ala	Gln	Val	Trp	Cys	Gly	Ser	Tyr	Arg
625					630					635					640
Pro	Glu	Phe	Ala	Ile	Gln	Ser	Ile	Lys	Thr	Asp	Val	His	Ser	Pro	Leu
				645					650						655
Lys	Tyr	Arg	Val	Leu	Gly	Ser	Leu	Gln	Asn	Leu	Ala	Ala	Phe	Ala	Asp
			660					665					670		
Thr	Phe	His	Cys	Ala	Arg	Gly	Thr	Pro	Met	His	Pro	Lys	Glu	Arg	Cys
		675					680					685			
Arg	Val	Trp													
		690													

<210> 3
 <211> 2340
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1) .. (2337)

<400> 3																
atg	ggg	aag	tcc	gaa	ggc	ccc	gtg	ggg	atg	gtg	gag	agc	gct	ggc	cgt	48
Met	Gly	Lys	Ser	Glu	Gly	Pro	Val	Gly	Met	Val	Glu	Ser	Ala	Gly	Arg	
1				5				10					15			
gca	ggg	cag	aag	cgc	ccg	ggg	ttc	ctg	gag	ggg	ggg	ctg	ctg	ctg	ctg	96
Ala	Gly	Gln	Lys	Arg	Pro	Gly	Phe	Leu	Glu	Gly	Gly	Leu	Leu	Leu	Leu	
			20				25					30				
ctg	ctg	ctg	gtg	acc	gct	gcc	ctg	gtg	gcc	ttg	ggt	gtc	ctc	tac	gcc	144
Leu	Leu	Leu	Val	Thr	Ala	Ala	Leu	Val	Ala	Leu	Gly	Val	Leu	Tyr	Ala	
			35				40					45				
gac	cgc	aga	ggg	aag	cag	ctg	cca	cgc	ctt	gct	agc	cgg	ctg	tgc	ttc	192
Asp	Arg	Arg	Gly	Lys	Gln	Leu	Pro	Arg	Leu	Ala	Ser	Arg	Leu	Cys	Phe	

50	55	60	
tta cag gag gag agg acc ttt gta aaa cga aaa ccc cga ggg atc cca	240		
Leu Gln Glu Glu Arg Thr Phe Val Lys Arg Lys Pro Arg Gly Ile Pro			
65 70 75 80			
gag gcc caa gag gtg agc gag gtc tgc acc acc cct ggc tgc gtg ata	288		
Glu Ala Gln Glu Val Ser Glu Val Cys Thr Thr Pro Gly Cys Val Ile			
85 90 95			
gca gct gcc agg atc ctc cag aac atg gac ccg acc acg gaa ccg tgt	336		
Ala Ala Ala Arg Ile Leu Gln Asn Met Asp Pro Thr Thr Glu Pro Cys			
100 105 110			
gac gac ttc tac cag ttt gca tgc gga ggc tgg ctg cgg cgc cac gtg	384		
Asp Asp Phe Tyr Gln Phe Ala Cys Gly Gly Trp Leu Arg Arg His Val			
115 120 125			
atc cct gag acc aac tca aga tac agc atc ttt gac gtc ctc cgc gac	432		
Ile Pro Glu Thr Asn Ser Arg Tyr Ser Ile Phe Asp Val Leu Arg Asp			
130 135 140			
gag ctg gag gtc atc ctc aaa gcg gtg ctg gag aat tcg act gcc aag	480		
Glu Leu Glu Val Ile Leu Lys Ala Val Leu Glu Asn Ser Thr Ala Lys			
145 150 155 160			
gac cgg ccg gct gtg gag aag gcc agg acg ctg tac cgc tcc tgc atg	528		
Asp Arg Pro Ala Val Glu Lys Ala Arg Thr Leu Tyr Arg Ser Cys Met			
165 170 175			
aac cag agt gtg ata gag aag cga ggc tct cag ccc ctg ctg gac atc	576		
Asn Gln Ser Val Ile Glu Lys Arg Gly Ser Gln Pro Leu Leu Asp Ile			
180 185 190			
ttg gag gtg gtg gga ggc tgg ccg gtg gcg atg gac agg tgg aac gag	624		
Leu Glu Val Val Gly Gly Trp Pro Val Ala Met Asp Arg Trp Asn Glu			
195 200 205			
acc gta gga ctc gag tgg gag ctg gag cgg cag ctg gcg ctg atg aac	672		
Thr Val Gly Leu Glu Trp Glu Leu Glu Arg Gln Leu Ala Leu Met Asn			
210 215 220			
tca cag ttc aac agg cgc gtc ctc atc gac ctc ttc atc tgg aac gac	720		
Ser Gln Phe Asn Arg Arg Val Leu Ile Asp Leu Phe Ile Trp Asn Asp			
225 230 235 240			
gac cag aac tcc agc cgg cac atc atc tac ata gac cag ccc acc ttg	768		
Asp Gln Asn Ser Ser Arg His Ile Ile Tyr Ile Asp Gln Pro Thr Leu			
245 250 255			
ggc atg ccc tcc cga gag tac tac ttc aac ggc ggc agc aac cgg aag	816		
Gly Met Pro Ser Arg Glu Tyr Tyr Phe Asn Gly Gly Ser Asn Arg Lys			
260 265 270			
gtg cgg gaa gcc tac ctg cag ttc atg gtg tca gtg gcc acg ttg ctg	864		
Val Arg Glu Ala Tyr Leu Gln Phe Met Val Ser Val Ala Thr Leu Leu			
275 280 285			
cgg gag gat gca aac ctg ccc agg gac agc tgc ctg gtg cag gag gac	912		
Arg Glu Asp Ala Asn Leu Pro Arg Asp Ser Cys Leu Val Gln Glu Asp			
290 295 300			

atg atg cag gtg ctg gag ctg gag aca cag ctg gcc aag gcc acg gta	960
Met Met Gln Val Leu Glu Leu Glu Thr Gln Leu Ala Lys Ala Thr Val	
305 310 315 320	
ccc cag gag gag aga cac gac gtc atc gcc ttg tac cac cgg atg gga	1008
Pro Gln Glu Glu Arg His Asp Val Ile Ala Leu Tyr His Arg Met Gly	
325 330 335	
ctg gag gag ctg caa agc cag ttt ggc ctg aag gga ttt aac tgg act	1056
Leu Glu Glu Leu Gln Ser Gln Phe Gly Leu Lys Gly Phe Asn Trp Thr	
340 345 350	
ctg ttc ata caa act gtg cta tcc tct gtc aaa atc aag ctg ctg cca	1104
Leu Phe Ile Gln Thr Val Leu Ser Ser Val Lys Ile Lys Leu Leu Pro	
355 360 365	
gat gag gaa gtg gtg gtc tat ggc atc ccc tac ctg cag aac ctt gaa	1152
Asp Glu Glu Val Val Val Tyr Gly Ile Pro Tyr Leu Gln Asn Leu Glu	
370 375 380	
aac atc atc gac acc tac tca gcc agg acc ata cag aac tac ctg gtc	1200
Asn Ile Ile Asp Thr Tyr Ser Ala Arg Thr Ile Gln Asn Tyr Leu Val	
385 390 395 400	
tgg cgc ctg gtg ctg gac cgc att ggt agc cta agc cag aga ttc aag	1248
Trp Arg Leu Val Leu Asp Arg Ile Gly Ser Leu Ser Gln Arg Phe Lys	
405 410 415	
gac aca cga gtg aac tac cgc aag gcg ctg ttt ggc aca atg gtg gag	1296
Asp Thr Arg Val Asn Tyr Arg Lys Ala Leu Phe Gly Thr Met Val Glu	
420 425 430	
gag gtg cgc tgg cgt gaa tgt gtg ggc tac gtc aac agc aac atg gag	1344
Glu Val Arg Trp Arg Glu Cys Val Gly Tyr Val Asn Ser Asn Met Glu	
435 440 445	
aac gcc gtg ggc tcc ctc tac gtc agg gag gcg ttc cct gga gac agc	1392
Asn Ala Val Gly Ser Leu Tyr Val Arg Glu Ala Phe Pro Gly Asp Ser	
450 455 460	
aag agc atg gtc aga gaa ctc att gac aag gtg cgg aca gtg ttt gtg	1440
Lys Ser Met Val Arg Glu Leu Ile Asp Lys Val Arg Thr Val Phe Val	
465 470 475 480	
gag acg ctg gac gag ctg ggc tgg atg gac gag gag tcc aag aag aag	1488
Glu Thr Leu Asp Glu Leu Gly Trp Met Asp Glu Glu Ser Lys Lys Lys	
485 490 495	
gcg cag gag aag gcc atg agc atc cgg gag cag atc ggg cac cct gac	1536
Ala Gln Glu Lys Ala Met Ser Ile Arg Glu Gln Ile Gly His Pro Asp	
500 505 510	
tac atc ctg gag gag atg aac agg cgc ctg gac gag gag tac tcc aat	1584
Tyr Ile Leu Glu Glu Met Asn Arg Arg Leu Asp Glu Glu Tyr Ser Asn	
515 520 525	
ctg aac ttc tca gag gac ctg tac ttt gag aac agt ctg cag aac ctc	1632
Leu Asn Phe Ser Glu Asp Leu Tyr Phe Glu Asn Ser Leu Gln Asn Leu	
530 535 540	

10/23

<210> 4

<211> 779

<212> PRT

<213> Homo sapiens

<400> 4

```

Met Gly Lys Ser Glu Gly Pro Val Gly Met Val Glu Ser Ala Gly Arg
 1             5             10             15

Ala Gly Gln Lys Arg Pro Gly Phe Leu Glu Gly Gly Leu Leu Leu Leu
          20             25             30

Leu Leu Leu Val Thr Ala Ala Leu Val Ala Leu Gly Val Leu Tyr Ala
      35             40             45

Asp Arg Arg Gly Lys Gln Leu Pro Arg Leu Ala Ser Arg Leu Cys Phe
      50             55             60

Leu Gln Glu Glu Arg Thr Phe Val Lys Arg Lys Pro Arg Gly Ile Pro
      65             70             75             80

Glu Ala Gln Glu Val Ser Glu Val Cys Thr Thr Pro Gly Cys Val Ile
          85             90             95

Ala Ala Ala Arg Ile Leu Gln Asn Met Asp Pro Thr Thr Glu Pro Cys
          100             105             110

Asp Asp Phe Tyr Gln Phe Ala Cys Gly Gly Trp Leu Arg Arg His Val
      115             120             125

Ile Pro Glu Thr Asn Ser Arg Tyr Ser Ile Phe Asp Val Leu Arg Asp
      130             135             140

Glu Leu Glu Val Ile Leu Lys Ala Val Leu Glu Asn Ser Thr Ala Lys
      145             150             155             160

Asp Arg Pro Ala Val Glu Lys Ala Arg Thr Leu Tyr Arg Ser Cys Met
          165             170             175

Asn Gln Ser Val Ile Glu Lys Arg Gly Ser Gln Pro Leu Leu Asp Ile
          180             185             190

Leu Glu Val Val Gly Gly Trp Pro Val Ala Met Asp Arg Trp Asn Glu
      195             200             205

Thr Val Gly Leu Glu Trp Glu Leu Glu Arg Gln Leu Ala Leu Met Asn
      210             215             220

Ser Gln Phe Asn Arg Arg Val Leu Ile Asp Leu Phe Ile Trp Asn Asp
      225             230             235             240

Asp Gln Asn Ser Ser Arg His Ile Ile Tyr Ile Asp Gln Pro Thr Leu
          245             250             255

Gly Met Pro Ser Arg Glu Tyr Tyr Phe Asn Gly Gly Ser Asn Arg Lys
          260             265             270

Val Arg Glu Ala Tyr Leu Gln Phe Met Val Ser Val Ala Thr Leu Leu
          275             280             285

Arg Glu Asp Ala Asn Leu Pro Arg Asp Ser Cys Leu Val Gln Glu Asp

```

290					295					300					
Met	Met	Gln	Val	Leu	Glu	Leu	Glu	Thr	Gln	Leu	Ala	Lys	Ala	Thr	Val
305					310					315					320
Pro	Gln	Glu	Glu	Arg	His	Asp	Val	Ile	Ala	Leu	Tyr	His	Arg	Met	Gly
				325					330					335	
Leu	Glu	Glu	Leu	Gln	Ser	Gln	Phe	Gly	Leu	Lys	Gly	Phe	Asn	Trp	Thr
			340					345					350		
Leu	Phe	Ile	Gln	Thr	Val	Leu	Ser	Ser	Val	Lys	Ile	Lys	Leu	Leu	Pro
		355					360					365			
Asp	Glu	Glu	Val	Val	Val	Tyr	Gly	Ile	Pro	Tyr	Leu	Gln	Asn	Leu	Glu
	370					375					380				
Asn	Ile	Ile	Asp	Thr	Tyr	Ser	Ala	Arg	Thr	Ile	Gln	Asn	Tyr	Leu	Val
385				390					395					400	
Trp	Arg	Leu	Val	Leu	Asp	Arg	Ile	Gly	Ser	Leu	Ser	Gln	Arg	Phe	Lys
				405				410						415	
Asp	Thr	Arg	Val	Asn	Tyr	Arg	Lys	Ala	Leu	Phe	Gly	Thr	Met	Val	Glu
			420				425						430		
Glu	Val	Arg	Trp	Arg	Glu	Cys	Val	Gly	Tyr	Val	Asn	Ser	Asn	Met	Glu
		435				440					445				
Asn	Ala	Val	Gly	Ser	Leu	Tyr	Val	Arg	Glu	Ala	Phe	Pro	Gly	Asp	Ser
	450				455						460				
Lys	Ser	Met	Val	Arg	Glu	Leu	Ile	Asp	Lys	Val	Arg	Thr	Val	Phe	Val
465				470					475					480	
Glu	Thr	Leu	Asp	Glu	Leu	Gly	Trp	Met	Asp	Glu	Glu	Ser	Lys	Lys	Lys
				485				490					495		
Ala	Gln	Glu	Lys	Ala	Met	Ser	Ile	Arg	Glu	Gln	Ile	Gly	His	Pro	Asp
			500					505					510		
Tyr	Ile	Leu	Glu	Glu	Met	Asn	Arg	Arg	Leu	Asp	Glu	Glu	Tyr	Ser	Asn
		515				520						525			
Leu	Asn	Phe	Ser	Glu	Asp	Leu	Tyr	Phe	Glu	Asn	Ser	Leu	Gln	Asn	Leu
	530				535					540					
Lys	Val	Gly	Ala	Gln	Arg	Ser	Leu	Arg	Lys	Leu	Arg	Glu	Lys	Val	Asp
545				550					555					560	
Pro	Asn	Leu	Trp	Ile	Ile	Gly	Ala	Ala	Val	Val	Asn	Ala	Phe	Tyr	Ser
				565				570					575		
Pro	Asn	Arg	Asn	Gln	Ile	Val	Phe	Pro	Ala	Gly	Ile	Leu	Gln	Pro	Pro
			580				585					590			
Phe	Phe	Ser	Lys	Glu	Gln	Pro	Gln	Ala	Leu	Asn	Phe	Gly	Gly	Ile	Gly
		595				600						605			
Met	Val	Ile	Gly	His	Glu	Ile	Thr	His	Gly	Phe	Asp	Asp	Asn	Gly	Arg
	610				615						620				

```
<220>  
<221> CDS  
<222> (1) .. (2259)
```

<400> 5																	
atg	ggg	aag	tcc	gaa	ggc	cca	gtg	ggg	atg	gtg	gag	agc	gcc	ggc	cgt		48
Met	Gly	Lys	Ser	Glu	Gly	Pro	Val	Gly	Met	Val	Glu	Ser	Ala	Gly	Arg		
1				5				10						15			
gca	ggg	cag	aag	cgc	ccg	ggg	ttc	ctg	gag	ggg	ggg	ctg	ctg	ctg	ctg		96
Ala	Gly	Gln	Lys	Arg	Pro	Gly	Phe	Leu	Glu	Gly	Gly	Leu	Leu	Leu	Leu		
		20						25						30			
ctg	ctg	ctg	gtg	acc	gct	gcc	ctg	gtg	gcc	ttg	ggt	gtc	ctc	tac	gcc		144
Leu	Leu	Leu	Val	Thr	Ala	Ala	Leu	Val	Ala	Leu	Gly	Val	Leu	Tyr	Ala		
		35						40						45			
gac	cgc	aga	ggg	atc	cca	gag	gcc	caa	gag	gtg	agc	gag	gtc	tgc	acc		192
Asp	Arg	Arg	Gly	Ile	Pro	Glu	Ala	Gln	Glu	Val	Ser	Glu	Val	Cys	Thr		
50						55						60					
acc	cct	ggc	tgc	gtg	ata	gca	gct	gcc	agg	atc	ctc	cag	aac	atg	gac		240
Thr	Pro	Gly	Cys	Val	Ile	Ala	Ala	Ala	Arg	Ile	Leu	Gln	Asn	Met	Asp		

65				70				75				80				
ccg Pro	acc Thr	acg Thr	gaa Glu	ccg Pro	tgt Cys	gac Asp	gac Asp	ttc Phe	tac Tyr	cag Gln	ttt Phe	gca Ala	tgc Cys	gga Gly	ggc Gly	288
				85					90					95		
tgg Trp	ctg Leu	cgg Arg	cgc Arg	cac His	gtg Val	atc Ile	cct Pro	gag Glu	acc Thr	aac Asn	tca Ser	aga Arg	tac Tyr	agc Ser	atc Ile	336
				100					105					110		
ttt Phe	gac Asp	gtc Val	ctc Leu	cgc Arg	gac Asp	gag Glu	ctg Leu	gag Glu	gtc Val	atc Ile	ctc Leu	aaa Lys	gcg Ala	gtg Val	ctg Leu	384
				115					120					125		
gag Glu	aat Asn	tgc Ser	act Thr	gcc Ala	aag Lys	gac Asp	cgg Arg	ccg Pro	gct Ala	gtg Val	gag Glu	aag Lys	gcc Ala	agg Arg	acg Thr	432
				130					135					140		
ctg Leu	tac Tyr	cgc Arg	tcc Ser	tgc Cys	atg Met	aac Asn	cag Gln	agt Ser	gtg Val	ata Ile	gag Glu	aag Lys	cga Arg	ggc Gly	tct Ser	480
				145					150					155		
cag Gln	ccc Pro	ctg Leu	ctg Leu	gac Asp	atc Ile	ttg Leu	gag Glu	gtg Val	gtg Val	gga Gly	ggc Gly	tgg Trp	ccg Pro	gtg Val	gcg Ala	528
				165					170					175		
atg Met	gac Asp	agg Arg	tgg Trp	aac Asn	gag Glu	acc Thr	gta Val	gga Gly	ctc Leu	gag Glu	tgg Trp	gag Glu	ctg Leu	gag Glu	cgg Arg	576
				180					185					190		
cag Gln	ctg Leu	gcg Ala	ctg Leu	atg Met	aac Asn	tca Ser	cag Gln	ttc Phe	aac Asn	agg Arg	cgc Arg	gtc Val	ctc Leu	atc Ile	gac Asp	624
				195					200					205		
ctc Leu	ttc Phe	atc Ile	tgg Trp	aac Asn	gac Asp	gac Asp	cag Gln	aac Asn	tcc Ser	agc Ser	cgg Arg	cac His	atc Ile	atc Ile	tac Tyr	672
				210					215					220		
ata Ile	gac Asp	cag Gln	ccc Pro	acc Thr	ttg Leu	ggc Gly	atg Met	ccc Pro	tcc Ser	cga Arg	gag Glu	tac Tyr	tac Tyr	ttc Phe	aac Asn	720
				225					230					235		
ggc Gly	ggc Gly	agc Ser	aac Asn	cgg Arg	aag Lys	gtg Val	cgg Arg	gaa Glu	gcc Ala	tac Tyr	ctg Leu	cag Gln	ttc Phe	atg Met	gtg Val	768
				245					250					255		
tca Ser	gtg Val	gcc Ala	acg Thr	ttg Leu	ctg Leu	cgg Arg	gag Glu	gat Asp	gca Ala	aac Asn	ctg Leu	ccc Pro	agg Arg	gac Asp	agc Ser	816
				260					265					270		
tgc Cys	ctg Leu	gtg Val	cag Gln	gag Glu	gac Asp	atg Met	atg Met	cag Gln	gtg Val	ctg Leu	gag Glu	ctg Leu	gag Glu	aca Thr	cag Gln	864
				275					280					285		
ctg Leu	gcc Ala	aag Lys	gcc Ala	acg Thr	gta Val	ccc Pro	cag Gln	gag Glu	gag Glu	aga Arg	cac His	gac Asp	gtc Val	atc Ile	gcc Ala	912
				290					295					300		
ttg Leu	tac Tyr	cac His	cgg Arg	atg Met	gga Gly	ctg Leu	gag Glu	gag Glu	ctg Leu	caa Gln	agc Ser	cag Gln	ttt Phe	ggc Gly	ctg Leu	960
				305					310					315		

aag gga ttt aac tgg act ctg ttc ata caa act gtg cta tcc tct gtc	1008
Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Thr Val Leu Ser Ser Val	
325 330 335	
aaa atc aag ctg ctg cca gat gag gaa gtg gtg gtc tat ggc atc ccc	1056
Lys Ile Lys Leu Leu Pro Asp Glu Glu Val Val Val Tyr Gly Ile Pro	
340 345 350	
tac ctg cag aac ctt gaa aac atc atc gac acc tac tca gcc agg acc	1104
Tyr Leu Gln Asn Leu Glu Asn Ile Ile Asp Thr Tyr Ser Ala Arg Thr	
355 360 365	
ata cag aac tac ctg gtc tgg cgc ctg gtg ctg gac cgc att ggt agc	1152
Ile Gln Asn Tyr Leu Val Trp Arg Leu Val Leu Asp Arg Ile Gly Ser	
370 375 380	
cta agc cag aga ttc aag gac aca cga gtg aac tac cgc aag gcg ctg	1200
Leu Ser Gln Arg Phe Lys Asp Thr Arg Val Asn Tyr Arg Lys Ala Leu	
385 390 395 400	
ttt ggc aca atg gtg gag gag gtg cgc tgg cgt gaa tgt gtg ggc tac	1248
Phe Gly Thr Met Val Glu Glu Val Arg Trp Arg Glu Cys Val Gly Tyr	
405 410 415	
gtc aac agc aac atg gag aac gcc gtg ggc tcc ctc tac gtc agg gag	1296
Val Asn Ser Asn Met Glu Asn Ala Val Gly Ser Leu Tyr Val Arg Glu	
420 425 430	
gcg ttc cct gga gac agc aag agc atg gtc aga gaa ctc att gac aag	1344
Ala Phe Pro Gly Asp Ser Lys Ser Met Val Arg Glu Leu Ile Asp Lys	
435 440 445	
gtg cgg aca gtg ttt gtg gag acg ctg gac gag ctg ggc tgg atg gac	1392
Val Arg Thr Val Phe Val Glu Thr Leu Asp Glu Leu Gly Trp Met Asp	
450 455 460	
gag gag tcc aag aag aag gcg cag gag aag gcc atg agc atc cgg gag	1440
Glu Glu Ser Lys Lys Lys Ala Gln Glu Lys Ala Met Ser Ile Arg Glu	
465 470 475 480	
cag atc ggg cac cct gac tac atc ctg gag gag atg aac agg cgc ctg	1488
Gln Ile Gly His Pro Asp Tyr Ile Leu Glu Glu Met Asn Arg Arg Leu	
485 490 495	
gac gag gag tac tcc aat ctg aac ttc tca gag gac ctg tac ttt gag	1536
Asp Glu Glu Tyr Ser Asn Leu Asn Phe Ser Glu Asp Leu Tyr Phe Glu	
500 505 510	
aac agt ctg cag aac ctc aag gtg ggc gcc cag cgg agc ctc agg aag	1584
Asn Ser Leu Gln Asn Leu Lys Val Gly Ala Gln Arg Ser Leu Arg Lys	
515 520 525	
ctt cgg gaa aag gtg gac cca aat ctc tgg atc atc ggg gcg gcg gtg	1632
Leu Arg Glu Lys Val Asp Pro Asn Leu Trp Ile Ile Gly Ala Ala Val	
530 535 540	
gtc aat gcg ttc tac tcc cca aac cga aac cag att gta ttc cct gcc	1680
Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Gln Ile Val Phe Pro Ala	
545 550 555 560	

```

ggg atc ctc cag ccc ccc ttc ttc agc aag gag cag cca cag gcc ttg 1728
Gly Ile Leu Gln Pro Pro Phe Phe Ser Lys Glu Gln Pro Gln Ala Leu
                    565                    570                    575

aac ttt gga ggc att ggg atg gtg atc ggg cac gag atc acg cac ggc 1776
Asn Phe Gly Gly Ile Gly Met Val Ile Gly His Glu Ile Thr His Gly
                    580                    585                    590

ttt gac gac aat ggc cgg aac ttc gac aag aat ggc aac atg atg gat 1824
Phe Asp Asp Asn Gly Arg Asn Phe Asp Lys Asn Gly Asn Met Met Asp
                    595                    600                    605

tgg tgg agt aac ttc tcc acc cag cac ttc cgg gag cag tca gag tgc 1872
Trp Trp Ser Asn Phe Ser Thr Gln His Phe Arg Glu Gln Ser Glu Cys
                    610                    615                    620

atg atc tac cag tac ggc aac tac tcc tgg gac ctg gca gac gaa cag 1920
Met Ile Tyr Gln Tyr Gly Asn Tyr Ser Trp Asp Leu Ala Asp Glu Gln
                    625                    630                    635                    640

aac gtg aac gga ttc aac acc ctt ggg gaa aac att gct gac aac gga 1968
Asn Val Asn Gly Phe Asn Thr Leu Gly Glu Asn Ile Ala Asp Asn Gly
                    645                    650                    655

ggg gtg cgg caa gcc tat aag gcc tac ctc aag tgg atg gca gag ggt 2016
Gly Val Arg Gln Ala Tyr Lys Ala Tyr Leu Lys Trp Met Ala Glu Gly
                    660                    665                    670

ggc aag gac cag cag ctg ccc ggc ctg gat ctc acc cat gag cag ctc 2064
Gly Lys Asp Gln Gln Leu Pro Gly Leu Asp Leu Thr His Glu Gln Leu
                    675                    680                    685

ttc ttc atc aac tac gcc cag gtg tgg tgc ggg tcc tac cgg ccc gag 2112
Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly Ser Tyr Arg Pro Glu
                    690                    695                    700

ttc gcc atc caa tcc atc aag aca gac gtc cac agt ccc ctg aag tac 2160
Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His Ser Pro Leu Lys Tyr
                    705                    710                    715                    720

agg gta ctg ggg tcg ctg cag aac ctg gcc gcc ttc gca gac acg ttc 2208
Arg Val Leu Gly Ser Leu Gln Asn Leu Ala Ala Phe Ala Asp Thr Phe
                    725                    730                    735

cac tgt gcc cgg ggc acc ccc atg cac ccc aag gag cga tgc cgc gtg 2256
His Cys Ala Arg Gly Thr Pro Met His Pro Lys Glu Arg Cys Arg Val
                    740                    745                    750

tgg tag
Trp

```

<210> 6

<211> 753

<212> PRT

<213> Homo sapiens

<400> 6

```

Met Gly Lys Ser Glu Gly Pro Val Gly Met Val Glu Ser Ala Gly Arg
 1              5              10              15

```

Ala Gly Gln Lys Arg Pro Gly Phe Leu Glu Gly Gly Leu Leu Leu Leu
 20 25 30
 Leu Leu Leu Val Thr Ala Ala Leu Val Ala Leu Gly Val Leu Tyr Ala
 35 40 45
 Asp Arg Arg Gly Ile Pro Glu Ala Gln Glu Val Ser Glu Val Cys Thr
 50 55 60
 Thr Pro Gly Cys Val Ile Ala Ala Ala Arg Ile Leu Gln Asn Met Asp
 65 70 75 80
 Pro Thr Thr Glu Pro Cys Asp Asp Phe Tyr Gln Phe Ala Cys Gly Gly
 85 90 95
 Trp Leu Arg Arg His Val Ile Pro Glu Thr Asn Ser Arg Tyr Ser Ile
 100 105 110
 Phe Asp Val Leu Arg Asp Glu Leu Glu Val Ile Leu Lys Ala Val Leu
 115 120 125
 Glu Asn Ser Thr Ala Lys Asp Arg Pro Ala Val Glu Lys Ala Arg Thr
 130 135 140
 Leu Tyr Arg Ser Cys Met Asn Gln Ser Val Ile Glu Lys Arg Gly Ser
 145 150 155 160
 Gln Pro Leu Leu Asp Ile Leu Glu Val Val Gly Gly Trp Pro Val Ala
 165 170 175
 Met Asp Arg Trp Asn Glu Thr Val Gly Leu Glu Trp Glu Leu Glu Arg
 180 185 190
 Gln Leu Ala Leu Met Asn Ser Gln Phe Asn Arg Arg Val Leu Ile Asp
 195 200 205
 Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser Ser Arg His Ile Ile Tyr
 210 215 220
 Ile Asp Gln Pro Thr Leu Gly Met Pro Ser Arg Glu Tyr Tyr Phe Asn
 225 230 235 240
 Gly Gly Ser Asn Arg Lys Val Arg Glu Ala Tyr Leu Gln Phe Met Val
 245 250 255
 Ser Val Ala Thr Leu Leu Arg Glu Asp Ala Asn Leu Pro Arg Asp Ser
 260 265 270
 Cys Leu Val Gln Glu Asp Met Met Gln Val Leu Glu Leu Glu Thr Gln
 275 280 285
 Leu Ala Lys Ala Thr Val Pro Gln Glu Glu Arg His Asp Val Ile Ala
 290 295 300
 Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln Ser Gln Phe Gly Leu
 305 310 315 320
 Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Thr Val Leu Ser Ser Val
 325 330 335
 Lys Ile Lys Leu Leu Pro Asp Glu Glu Val Val Val Tyr Gly Ile Pro

340	345	350
Tyr Leu Gln Asn Leu Glu Asn Ile Ile Asp Thr Tyr Ser Ala Arg Thr		
355	360	365
Ile Gln Asn Tyr Leu Val Trp Arg Leu Val Leu Asp Arg Ile Gly Ser		
370	375	380
Leu Ser Gln Arg Phe Lys Asp Thr Arg Val Asn Tyr Arg Lys Ala Leu		
385	390	395
Phe Gly Thr Met Val Glu Glu Val Arg Trp Arg Glu Cys Val Gly Tyr		
	405	410
		415
Val Asn Ser Asn Met Glu Asn Ala Val Gly Ser Leu Tyr Val Arg Glu		
	420	425
		430
Ala Phe Pro Gly Asp Ser Lys Ser Met Val Arg Glu Leu Ile Asp Lys		
	435	440
		445
Val Arg Thr Val Phe Val Glu Thr Leu Asp Glu Leu Gly Trp Met Asp		
	450	455
		460
Glu Glu Ser Lys Lys Lys Ala Gln Glu Lys Ala Met Ser Ile Arg Glu		
465	470	475
		480
Gln Ile Gly His Pro Asp Tyr Ile Leu Glu Glu Met Asn Arg Arg Leu		
	485	490
		495
Asp Glu Glu Tyr Ser Asn Leu Asn Phe Ser Glu Asp Leu Tyr Phe Glu		
	500	505
		510
Asn Ser Leu Gln Asn Leu Lys Val Gly Ala Gln Arg Ser Leu Arg Lys		
	515	520
		525
Leu Arg Glu Lys Val Asp Pro Asn Leu Trp Ile Ile Gly Ala Ala Val		
	530	535
		540
Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Gln Ile Val Phe Pro Ala		
545	550	555
		560
Gly Ile Leu Gln Pro Pro Phe Phe Ser Lys Glu Gln Pro Gln Ala Leu		
	565	570
		575
Asn Phe Gly Gly Ile Gly Met Val Ile Gly His Glu Ile Thr His Gly		
	580	585
		590
Phe Asp Asp Asn Gly Arg Asn Phe Asp Lys Asn Gly Asn Met Met Asp		
	595	600
		605
Trp Trp Ser Asn Phe Ser Thr Gln His Phe Arg Glu Gln Ser Glu Cys		
	610	615
		620
Met Ile Tyr Gln Tyr Gly Asn Tyr Ser Trp Asp Leu Ala Asp Glu Gln		
625	630	635
		640
Asn Val Asn Gly Phe Asn Thr Leu Gly Glu Asn Ile Ala Asp Asn Gly		
	645	650
		655
Gly Val Arg Gln Ala Tyr Lys Ala Tyr Leu Lys Trp Met Ala Glu Gly		
	660	665
		670

Gly Lys Asp Gln Gln Leu Pro Gly Leu Asp Leu Thr His Glu Gln Leu
 675 680 685
 Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly Ser Tyr Arg Pro Glu
 690 695 700
 Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His Ser Pro Leu Lys Tyr
 705 710 715 720
 Arg Val Leu Gly Ser Leu Gln Asn Leu Ala Ala Phe Ala Asp Thr Phe
 725 730 735
 His Cys Ala Arg Gly Thr Pro Met His Pro Lys Glu Arg Cys Arg Val
 740 745 750
 Trp

<210> 7
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Reverse Primer

<400> 7
 acacggcatc gctccttg

18

<210> 8
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Degenerated
 Forward Primer

<400> 8
 ccccttgac ggtgaaygcn twyta

25

<210> 9
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Reverse Primer

<400> 9
 aatccgttca cgttctgttc gtctgcc

27

<210> 10
 <211> 24
 <212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Degenerated
Forward Primer

<400> 10

cctggaggag ctgvhntgga tgra

24

<210> 11

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Adaptor Primer

<400> 11

ccatccta at acgactcact atagggc

27

<210> 12

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 12

gtccttgcca ccctctgcca tcc

23

<210> 13

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 13

accacccccg ccccgatgat ccagag

26

<210> 14

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Specific
Anti-Sense Primer/Reverse Oligonucleotide Primer

<400> 14

acagccggct agcaaggcgt ggcagctg

28

<210> 15

<211> 28

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Specific
Anti-Sense Primer

<400> 15
acgacagccg gctagcaagg cgtggcag 28

<210> 16
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Degenerated
Forward Primer

<400> 16
ggncatsatgg tncttsctst scts 24

<210> 17
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Specific
Forward Primer

<400> 17
ctcctgagtg agcaaagggt cc 22

<210> 18
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Specific
Reverse Primer

<400> 18
gcaaactggt agaagtcgtc acac 24

<210> 19
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 19
gacaaggcct attatgccga gatcgtgctg cagccgctcg 40

<210> 20
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 20
aaggccagca acagggcccc cgagcggtg cagcacgatc 40

<210> 21
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 21
.ggggccctgt tgctggcctt gctgcttcaa gcctccatgg 40

<210> 22
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 22
gtgagaaccg ccacgcactt ccatggaggc ttgaagcagc 40

<210> 23
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 23
aagtgcgtgg cggttctcac catcaccacc atcacagcga 40

<210> 24
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 24
agccaggggt ggtgcagacc tcgctgtgat ggtggtgatg 40

<210> 25
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 25
gggtctgcacc acccctggct gcgtgatagc agctgccagg 40

<210> 26
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 26
gggtccatgt tctggaggat cctggcagct gctatcacgc 40

<210> 27
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 27
gacaaggcct attatg 16

<210> 28
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 28
gggtccatgt tctg 14

<210> 29
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 29
agcgaggtct gcac 14

<210> 30

<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 30
gtagatgatg tgccg

15

<210> 31
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 31
gcactagtct tggctaccac acgcggcatc gctccttg

38

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/11532

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12N9/64 C12N15/57 A61K38/48 C12Q1/68 G01N33/50 C07K16/40 C12Q1/37 A61K48/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) STRAND, WPI Data, PAJ, EPO-Internal, BIOSIS, CHEM ABS Data, SCISEARCH, BIOTECHNOLOGY ABS, MEDLINE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 777 291 A (INST NAT SANTE RECH MED) 15 October 1999 (1999-10-15) * the whole document, especially pages 12-16 and claim 2 * --- -/--	1,4,7-9, 13, 15-21, 23-26,28
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.		
<input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *8* document member of the same patent family		
Date of the actual completion of the international search 10 April 2001		Date of mailing of the international search report 20/04/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016		Authorized officer De Kok, A

INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/EP 00/11532

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOJI IKEDA ET AL: "Molecular identification and characterization of novel membrane-bound metalloprotease, the soluble secreted form of which hydrolyzes a variety of vasoactive peptides" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 45, 5 November 1999 (1999-11-05), pages 32469-32477, XP002140284 BALTIMORE US ISSN: 0021-9258 the whole document	1-17
Y	WO 92 13944 A (BERLEX LAB) 20 August 1992 (1992-08-20) page 4, line 11 - line 23 page 22 -page 30	1
A		19,20,23
Y	VALDENNAIRE O ET AL: "XCE, A NEW MEMBER OF THE ENDOTHELIN-CONVERTING ENZYME AND NEUTRAL ENDOPEPTIDASE FAMILY, IS PREFERENTIALLY EXPRESSED IN THE CNS" MOLECULAR BRAIN RESEARCH,NL,ELSEVIER SCIENCE BV, AMSTERDAM, vol. 64, no. 2, 1999, pages 211-221, XP000863086 ISSN: 0169-328X * the whole document, especially page 212-213 *	1
A		7
A	EP 0 596 355 A (GENENTECH INC) 11 May 1994 (1994-05-11) * the whole document, especially figure 1 *	1-4, 6-11, 13-17, 24-26
A	WO 89 05353 A (DANA FARBER CANCER INST INC) 15 June 1989 (1989-06-15) * the whole document, especially figure 3 and claim 4 *	1,6,7, 14-18, 23-26
P,X	WO 00 47750 A (UNIVERSITE DE MONTREAL (CA)) 17 August 2000 (2000-08-17) the whole document, especially page 7	1-21, 23-28
E	EP 1 069 188 A (SANOFI SYNTHELABO) 17 January 2001 (2001-01-17) the whole document, especially seq.id.no.1	1-21, 23-28

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**Continuation of Box I.1**

Although claim 24 and claim 28, first sentence, are directed to a diagnostic method practised on the human/animal body, the search has been carried out based on the assumption that the method has been carried out on a sample derived from the human/animal body.

Although claims 25, 26 and 28, second sentence, are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds, as far as they do not relate to stimulators respectively inhibitors or competing or degrading polypeptides of IGS5

Continuation of Box I.2

Claims Nos.: 22, 23a, 23b, 25a, 26a, 26c-e, 27

Present claims 22, 23a, 23b, 25a, 26a, 26c-e and 27 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for NONE of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, NO search has been carried out for those claims.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/EP 00/11532

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2777291 A	15-10-1999	EP 1068336 A WO 9953077 A	17-01-2001 21-10-1999
WO 9213944 A	20-08-1992	CA 2100355 A EP 0575405 A	05-08-1992 29-12-1993
EP 0596355 A	11-05-1994	US 4960700 A AT 119936 T AU 616876 B AU 8305787 A AU 623845 B AU 8305887 A DE 3751169 D DE 3751169 T DK 684087 A EP 0272928 A EP 0272929 A ES 2072251 T IE 66333 B IL 84928 A JP 1172344 A JP 2685468 B NZ 223028 A US 5780025 A CA 1322160 A DE 3751748 D DE 3751748 T DK 684487 A IL 84929 A NZ 223029 A ZA 8709605 A ZA 8709606 A	02-10-1990 15-04-1995 14-11-1991 30-06-1988 28-05-1992 30-06-1988 20-04-1995 26-10-1995 03-10-1988 29-06-1988 29-06-1988 16-07-1995 27-12-1995 27-02-1994 07-07-1989 03-12-1997 26-09-1990 14-07-1998 14-09-1993 25-04-1996 14-11-1996 07-10-1988 04-04-1993 26-02-1991 30-08-1989 30-08-1989
WO 8905353 A	15-06-1989	AU 2801089 A EP 0391953 A JP 3505963 T ZA 8808963 A	05-07-1989 17-10-1990 26-12-1991 30-08-1989
WO 0047750 A	17-08-2000	AU 2653400 A	29-08-2000
EP 1069188 A	17-01-2001	NONE	